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EXAMINATION OF *ORNITHOBACTERIUM RHINOTRACHEALE* PRESENCE AND PATHOMORPHOLOGICAL CHANGES IN BROILER RESPIRATORY ORGANS IN INTENSIVE BROILER PRODUCTION

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Three flocks of broilers from the epizootiological region of South Banat, Serbia were chosen for examination, one flock with manifestation of respiratory disorders and two control flocks without respiratory disorders. In the flock with manifested respiratory disorders which was marked as flock number one (flock 1), high seroprevalence of specific antibodies for O. rhinotracheale (46%) was found in 30-day-old broiler chickens. The symptoms presented were: depression, reduction in feed consumption and water intake, sneezing, mucosal nasal discharge and facial edema. Production results were below technological standards due to increased mortality, decreased growth range and increased number of stunted chickens. Autopsy found frequent changes in the trachea, air sacs and lungs, which were manifested as: tracheitis catarrhalis, aerosacculitis fibrinosa and pneumonia fibrinosa.

In control flocks marked as flock number two (flock 2) and flock number three (flock 3) where 4 % and 8 % of seropositive chickens were found, there were no clinical signs which would indicate illness, and production results were much closer to technological standards as compared with flock 1. Autopsy found lower number of chickens with changed respiratory organs as compared with flock 1; in addition intensity of changes was lower than in flock 1. No case of airsacculitis was found in flocks 2 and 3, which was a frequent finding in flock 1.

Key words: broiler, Ornithobacterium rhinotracheale, pathomorphological changes, respiratory organs

INTRODUCTION

Ornithobacterium rhinotracheale is a relatively recently discovered bacterium and its role in the complex group of poultry respiratory diseases is still being researched. The first case of the disease in broilers, caused by this microorganism, which manifested itself with mild respiratory symptoms, was observed in 1991 by Jan du Preez in South Africa (Van Beek *et al*., 1994). Gram-

negative, pleomorphic, slow growing rod bacterium, discovered by bacteriological examination of deceased birds' organs, could not be classified as any of the known bacterial species, The actual name was accepted and the agent was classified in 1994 (Vandamme *et al*., 1994). Since then, this bacterium has been isolated worldwide from organs of birds which showed respiratory signs (van Empel and Hafez, 1999; Asadpour *et al*., 2008; Tabatabai *et al*., 2010; Gornatti Churria *et al*., 2011).

Considering the importance of respiratory diseases in modern broiler industry, their complex etiology, need of rapid and reliable diagnostics and the fact that presence of *O. rhinotracheale* wasn't discovered in the Republic of Serbia at the moment of these investigations, we decided to explore the presence of this bacterium (indirectly-serologically) and its influence on pathomorphological changes in broilers. The aim of the investigations was to determine the presence of infection caused by *O. rhinotracheale* in poultry in intensive broiler production in the epizootiological area of South Banat, its clinical manifestations, and gross and pathohistological changes in the respiratory organs.

Three flocks of broilers were chosen for testing: one flock with manifestated respiratory disorders and two control flocks without respiratory disorders. In chosen flocks the following investigations were carried out: health monitoring, examination of gross changes and microscopical changes of macroscopically changed respiratory organs and examination of the presence of specific antibodies to *O. rhinotracheale.*

MATERIAL AND METHODS

Animals

Target flock, flock 1 was selected on the basis of pathomophological examination of chickens that died, because in fifteen-day-old chickens, we detected airsacculitis which was manifested with presence of liquid, foamy, yellow-white content in the air sacs. Control flocks, were chosen on the basis of history data from which it was known that the percentage of mortality was below the technological limit and that until the beginning of testing none of the clinical symptoms of the disease were observed. All three flocks were examined from the fifteenth day of age until the end of the fattening period, which lasted 42 days. Data about production results were available from the first day of age. Flock 1 consisted of 7983 broiler chickens of both sexes, of "Cobb 500" provenience, flock 2 consisted of 11958 broiler chickens of both sexes, of "Cobb 500" provenience and flock 3 consisted of 11535 broiler chickens of both sexes of "Hubbard classic" provenience. All three flocks were raised on deep litter in similar environmental conditions. Chickens were fed *ad libitum* with complete feed mixtures with balanced nutrient content adjusted to their growing needs. In the first three weeks broilers were fed with a mixture 1 ("starter") with 22% raw protein and 13.0 MJME/kg, from 22^{nd} to 35^{th} day with mixture 2 ("grover") with 19% raw protein and 13.0 MJME/kg, and from 36^{th} to 42^{nd} day with mixture 3 ("finisher") with 17% raw protein and 13.0 MJME/kg. The population density was 15 chickens per m^2.

Sampling

For every three to five days, health condition was observed by adspection and post-mortem examination of chickens was performed. Tissue samples of macroscopically changed organs (lungs and trachea) were taken and subjected to pathohistological examination. Total of 180 broiler corpses were autopsied (60 corpses per flock) and 23 tissue samples of lungs and trachea from flock 1 were taken, from flock 2 six tissue samples of lungs and trachea were taken, and from flock 3 four tissue samples of lungs and trachea were taken. Blood samples from thirty-day-old chickens were taken by cardiopunction and serum was separated to determine the presence of *O. rhinotracheale* specific antibody titre; 100 blood samples per flock were examined (a total of 300 samples).

Serological examination

Samples of blood serum were examined by ELISA (enzyme-linked immunosorbent assay) according to manufacturer's instructions. For the above testing ELISA diagnostic kit "Flock Chek ORT" produced by IDEXX was used.

Pathohistological examination

For pathohistological examination tissue samples of lungs and trachea were taken and fixed in 10% buffered formalin. After standard processing in an automated tissue processor the samples were embedded in paraffin blocks. Approximately 5 μm thick paraffin sections were stained using standard hematoxylin-eozin method (HE).

RESULTS

Health condition for flock 1

Monitoring of health condition started in fifteen-day-old chickens, when an increased number of stunted chickens was noticed in the flock. Mortality increased in relation to the technological norm during the third week when it increased to 1.5%. At the end of the third week the signs of depression and decreased food and water intake appeared. The average weight of chickens at three weeks of age was 526 g (technological norm is 930 g). First respiratory symptoms appeared at the beginning of the fourth week (23rd day), and manifested with mucous nasal discharge and sneezing. Within a small number of infected chickens facial edema was noticed. The average weight of chickens at 28 days was 917 g (technological norm is 1467 g).

Macroscopical findings for flock 1

After observing the first case of airsacculitis in a fifteen-day-old broiler chick, the autopsy was done every three to five days until the end of the fattening period (up to 42nd day). Macroscopically visible changes in the respiratory organs were found in 23 corpses. The changes were most frequent in the trachea, air sacs and lungs.

At the beginning of the disease, in the third week of age, tracheal mucosa of several chickens was more or less reddened, with mucus accumulation on its

surface. The walls of air sacs were slightly opaque. There was a watery, transparent content or a watery, foamy, yellowish content in them. The most characteristic finding was in one broiler showing a collection of foamy, yellowish, yogurt-like consistence exudate in the thoracic (caudal) and abdominal air sacs (Figure 1). The affected lungs at this age were enlarged, firm, dark red, with blunt edges and foamy-hemorrhagic fluid on the dissected surface.

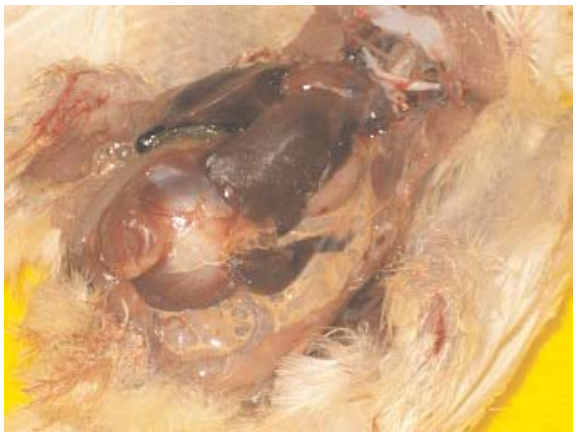

Figure 1. Airsacculitis in three-week-old broiler chick. Thoracic and abdominal air sacs are filled with yellowish, foamy, yogurt-like consistence exudate

In the fourth week of age, changes in the trachea were characterized by redness of the mucosa and the presence of mucous content in lumen. Such noticeable changes were present in older chickens, too. The walls of the air sacs were thickened, with firm content of white-yellow to grayish-yellow color attached to them. In some chickens changes were seen only in the thoracic or abdominal, and in others both thoracic and abdominal air sacs. Most of the changed lungs of chickens that died at this age were enlarged, firm, dark red, with blunt edges. The dissected surface of thus changed lungs was smooth, dimly-red to grayish-red. In most cases the changes were manifested in both lungs, although in some cases only the left lung was changed.

In the five and six-week-old chickens changes were more frequent in the thoracic than in the abdominal air sacs. The changes were very expressed in the thoracic caudal air sacs which were filled with great amount of white-yellow to yellow-gray, solid content which in some cases completely filled the lumen of air sacs. Changes in the lungs in this age group were similar to those in the previous period, except for the presence of the whitish-yellow patchy deposits, of up to two millimeters in thickness was noticed on the surface of the lungs (Figure 2).

The affected lungs were consolidated, dark-red in color. The changes were manifested in both lungs while in one chick (five weeks old) only the left lung was affected. In some corpses, at six weeks of age, the pericardium, as well as liver serosa was affected with fibrinous inflammation. In these cases, on the liver

surface could be seen whitish to whitish-yellow deposits up to two millimeters thick that could be easily removed from the organ surface. On the pericardial surface and in the pericardial cavity, there were also whitish-yellow fibrin deposits, and in one case the entire pericardium was wrapped with such content in the form of a shield.

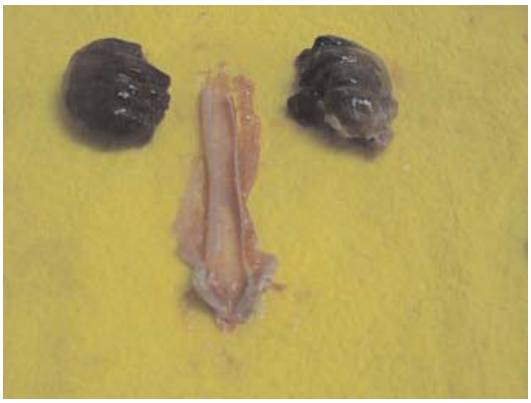

Figure 2. Pneumonia in five-week-old broiler chick. Enlarged, dark-red, firm lungs with fibrin deposits on the surface of the left lung. Tracheal mucosa is reddened

Within 23 corpses with changed respiratory organs, *hyperaemia mucosae tracheae* was found in 4 corpses, 7 were with *tracheitis catarrhalis*; 17 corpses were with *aerosacculitis* (4 of them had *aerosacculitis thoracalis et abdominalis*, 7 had *aerosacculitis thoracalis*, and 6 had *aerosacculitis abdominalis*); within 10 corpses the finding was *hyperaemia pulmonum*, and 2 of those 10 also had *oedema pulmonum*; 12 corpses were with *pneumonia fibrinosa*, one carcass with *haemorrhagiae pulmonum*; four corpses with *pleuritis fibrinosa*; 3 with *peritonitis* (two of them-*peritonitis serosa*, and one with-*peritonitis serofibrinosa*); 4 corpses had *pericarditis fibrinosa*; and final 3 had *perihepatitis fibrinosa*.

Microscopical findings for flock 1

Macroscopically changed respiratory organs (lungs and trachea) of 23 broiler corpses were examined pathohistologically. Pathohistological finding in the trachea of the majority of samples was catarrhal inflammation. Trachea blood vessels were filled with blood, the epithelium was thickened, and in subepithelial tissue was a cellular infiltrate with domination of heterophiles. The lumen was filled with a certain amount of mucous to mucous-cell exudate (Figure 3).

In some samples, described changes were accompanied with epithelium desquamation. In the larger diameter air ways accumulation of mucous content with heterophils was found. Dominant pathohistological finding in the lungs was *pneumonia fibrinosa*. Depending on duration of the process in the lungs of individual corpses different stages of inflammatory process could be seen (Figure 4).

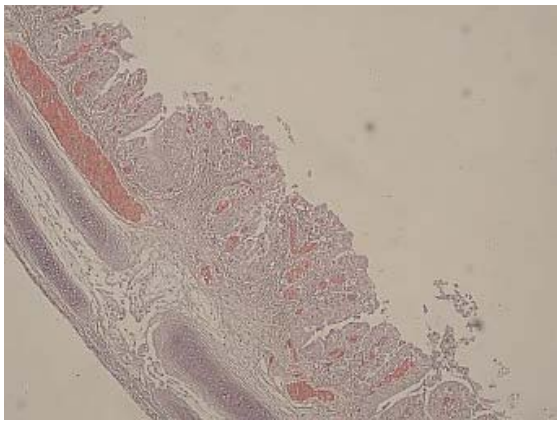

Figure 3. Tracheitis in 30-day-old broiler chick. The epithelium is thickened, tracheal wall is hyperemic and infiltrated by heterophiles, while the lumen is filled with small amount of mucus (HE, obj. 10 x)

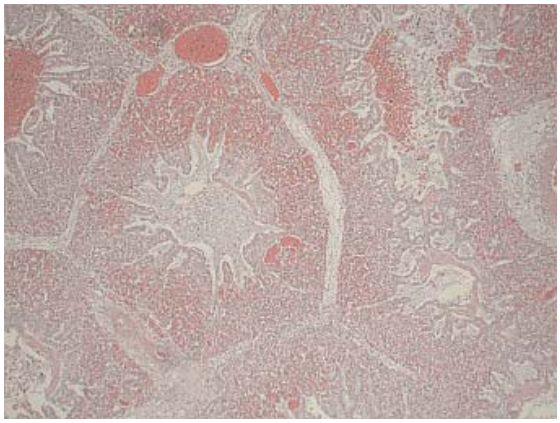

Figure 4. Pneumonia in 22-day-old broiler chick. Parabronchi and atria are filled with fibrin and erytrocytes (HE, obj. 10 x)

In one case, granulomatous inflammation was observed in the lungs. Around the necrotic center concentric layers of epitheloid cells, lymphocytes and fibroblasts were arranged.

Generally, changes in the lungs were characterized by fibrinous inflammation, while the amount of collected exudate varied in some samples from minor amounts to obturation of air ways. In a number of broilers the inflammation was so expressed that the air ways (bronchi, parabronchi and atria) were completely obturated with fibrinous-cell exudate.

Serological examination for flock 1

Out of 100 examined serum samples, using ELISA, 46 samples reacted positive. Titer values of positive samples were in the interval from 1016 to 5427, the average value was 2310.

Monitoring of some production parameters for flock 1

Fattening period lasted 42 days. During fattening 748 (9.37%) chickens died while 57 chickens (0.71%) were stunted. The average weight at the end of fattening was 1.64 kg per chick (technological norm 2.63 kg) and food conversion was 2.0 (technological norm 1.76).

Health condition for control flocks

During the investigation period from the 15th to 42nd day of fattening, broilers from flock 2 and flock 3 had harmonious body structure, properly developed bone and muscle tissue, a lively temperament and good overall condition. No visible changes were found on the skin, feathers and visible mucous membranes. Appetite during the entire fattening was good, and feces were normally formed. The ability of active movement and coordination of movements was good, and muscle tone was normally expressed.

Patomorphological analysis for control flocks

In broilers from flock 2 macroscopically visible changes in respiratory organs were found in six corpses. In five corpses changes in trachea were found: in four corpses *tracheitis*, and in one corpse *necrosis mucosae tracheae*. Changes in the lungs were found in six corpses, in four corpses *pneumonia fibrinosa*, and in two corpses *hyperaemia pulmonum*.

Pathohistological examination of macroscopically changed organs has shown hyperemia of the tracheal mucosa in four corpses. In two of these corpses there was mucous-desqumative exudate and in one corpse epithelial proliferation was found. The tracheal mucosa of one corpse was necrotic. In four broiler corpses in parabronchi and atria, there were found fibrin, erytrocyites and leukocytes. Lungs of one broiler corpse were hyperemic and with small amount of mucus in parabronchi. In the lungs of one chick which were hyperemic, erytrocytes were also found in the lumen of the parabronchi.

In broilers from flock 3 macroscopically visible changes in respiratory organs were found in 4 corpses: in 2 corpses *tracheitis*, in 3 corpses *hyperemia pulmonum* and in one corpse *pneumonia*.

Pathohistological examination of macroscopically changed organs found hyperemia of the tracheal mucosa, epithelial hyperplasia and necrosis in two corpses. Lungs were hyperemic, in the parabronchi and atria of two chickens, serous to serous-cell exudate was found. In one set of lungs parabronchi were filled with fibrin and cell detritus.

Serological examinations for control flocks

Out of 100 examined blood serum samples of broilers from flock 2, using ELISA 4 samples reacted positively. Titer values of positive samples were in the

interval from 893 to 1979, the average value was 1653. Out of 100 examined blood serum samples of broilers from flock 3, eight samples reacted positive. Titer values of positive samples were in the interval from 1014 to 2356, the average value was 1566.

Monitoring of some production parameters for control flocks

During the fattening 88 chickens (0.74%) from flock 2 died while 114 chickens (0.95%) were stunted. The average weight at the end of fattening was 2.09 kg per chick (technological norm 2.63 kg) and food conversion was 1.96 (the technological norm 1.76). In flock 3, 609 chickens (5.28%) died while 35 chickens were stunted (0.30%). The average weight at the end of fattening was 2.20 kg per chick (technological norm 2.59 kg) and food conversion was 1.92 (the technological norm 1.74).

DISCUSSION

A number of researchers in various countries dealt with the examination of the presence of *O. rhinotracheale* by serological methods. Although data on the percentage of seropositive flocks and seropositive samples of some authors vary, the results clearly indicate that the agent is very widespread in intensive broiler industry (Hafez and Sting, 1996; Ryll *et al*., 1997; Canal *et al*., 2003; Chansiripornchai *et al*., 2007; Suzuki *et al*., 2010; Uriarte *et al*., 2010).

In flock 1, clinical manifestations were similar to literature data. Clinical signs in broiler chickens generally appear between the third and sixth week of age with a mortality rate of 2 to 10% and with the following symptoms: depression, nasal discharge, sneezing, facial edema, decreased food intake and reduced weight gains (van Beek *et al*., 1994; Chin *et al*., 2003; Gornatti Churria *et al*., 2011).

Autopsy of 60 broiler corpses from flock 1, at different ages, found macroscopically visible changes in respiratory organs of 23 corpses. The most characteristic were changes in the air sacs and lungs. In the air sacs of the majority chickens with lesions in the respiratory organs a fibrinous exudate was found. At the beginning of the disease (in younger chickens) the exudate was foamy, whitish-yellow, similar to yogurt. Similar finding was described characteristic for *O. rhinotracheale* infection by some authors (Odor *et al*., 1997; van den Bosch, 2001; Gornatti Churria *et al*., 2011). In the air sacs of infected broilers can be seen a foamy, white, yogurt-like exudate, commonly accompanied with unilateral pneumonia. These lesions may disappear within one week, and could also be masked by other advancing infections and therefore not recognizable as *O. rhinotracheale* infection any more (van den Bosch, 2001). During our investigations only the youngest dissected corpses were found to have changes in the air sacs in appearance of watery, whitish-yellow, foamy content while in the later period there were firm fibrin deposits in the air sacs. Odor *et al*. (1997) described cases of concomitant infections challenged with *O. rhinotracheale* and infectious bronchitis virus (IBV) on the Delmarva Peninsula, USA when a macroscopical finding in air sacs was characterized by profuse, yellow to white, foamy content which contained floating "islands" of caseous

debris. De Rosa *et al*. (1996) described macroscopical changes in infected turkey breeders in USA. Lungs were enlarged, firm, congested and had a fibrinous exudate on pleura. The walls of air sacs were severely thickened because of the fibrinous exudate while a few birds had caseous exudate within the lumen of the air sacs. These lesions are consistent with lesions which we found in broiler corpses, 5-6 weeks of age from flock 1.

Microscopic lesions observed in the lungs of broilers from flock 1 are very similar to those discribed by: De Rosa *et al*. (1996), Sprenger *et al*. (1998), Chin *et al*. (2003), Gornatti Churria *et al*. (2011) although these authors didn't find erytrocytes in the air ways, which was a frequent finding in our investigation. The explanation of that could be found in the fact that tissue samples for pathohistological examination were frequently taken since the 15th day of age so the early stage of fibrinous pneumonia could be detected. Pathohistological finding in broilers from flock 1 wasn't completely in accordance to changes described by Odor *et al*. (1997) and van Veen *et al*. (2000) in concomitant infections challenged with *O. rhinotracheale* and IBV or *O. rhinotracheale* and Newcastle disease virus. Diffuse lymphocyte infiltration of the lungs and trachea could be explained as a response of the organism to viral infection while fibrinous to fibrino-purulent changes appeared due to *O. rhinotracheale* infection.

Production results for flock 1 in which 46% seropositive samples were detected were considerably under technological norms. The literature states that with other signs *O. rhinotracheale* infection is accompanied by decreased weight gain (van Empel *et al*., 1996; van Empel and Hafez, 1999; Chin *et al*., 2003).

In two control flocks, with low seroprevalence of specific antibodies for *O. rhinotracheale,* there were no clinical signs and autopsy found a lower number of chickens with lesions in the respiratory organs. Although exact etiology of these lesions wasn't determined, they were considerably less frequent and extensive as compared with lesions in broilers from flock 1. No case of airsacculitis was found in flocks 2 and 3, which was frequent finding in flock 1 and which is considered characteristic for *O. rhinotracheale* infection according to literature data (Odor *et al*., 1997; van den Bosch, 2001; Gornatti Churria *et al*., 2011). Production results in control flocks were much closer to technological standards comparing to flock 1. These deviations of control flocks are result of the production practices which rarely completely comply with production standards in area of breeding.

Our investigations determined that *O. rhinotracheale* was spread in intensive broiler production in the region of South Banat, Serbia, even though its presence did not always lead to clinical manifestation of respiratory tract disorders. The fact that the presence of seropositive chickens was determined in all of the three examined flocks, but with clinical and pathomorphological manifestation only in flock 1 could explain character of infection caused by *O. rhinotracheale.* Reasons for clinical appearance of infection could be found in influence of environmental factors and influence of other microorganisms which were not the subject of our investigations.

In the infected flock in which high seroprevalence of specific antibodies was detected; following clinical symptoms appeared in the form of depression, reduction in feed consumption and water intake, sneezing, mucosal nasal

discharge and facial edema. Production results were badly affected by the disease, leading to decreased growth range and increased number of stunted chickens. The most frequent pathomorphological changes were in trachea, air sacs and lungs, and were manifested as: *tracheitis catarrhalis, aerosacculitis fibrinosa and pneumonia fibrinosa.*

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ISPITIVANJE PRISUSTVA ORNITHOBACTERIUM RHINOTRACHEALE I PATOMORFOLOŠKIH PROMENA U RESPIRATORNIM ORGANIMA BROJLERA U INTENZIVNOJ BROJLERSKOJ PROIZVODNJI

GAVRILOVIĆ P, JOVANOVIĆ M i ŽIVULJ A

SADRŽAJ

Za ispitivanje su odabrana tri jata brojlera sa epizootiološkog područja Južnobanatskog okruga: jato sa manifestnim respiratornim poremećajem i dva kontrolna jata bez respiratornih poremećaja. U jatu sa manifestnim respiratornim poremećajem koje je označeno kao jato broj jedan (jato 1), u tridesetom danu starosti pilića utvrđena je visoka seroprevalencija specifičnih antitela za *O. rhinotracheale* (46 %). Od simptoma su bili prisutni: depresija, smanjeno konzumiranje hrane i vode, kijanje, sluzavi iscedak iz nosa i edem u predelu lica. Proizvodni rezultati su bili ispod tehnoloških normativa zbog povišenog mortaliteta, smanjenog prirasta i povećanog broja kržljavaca u jatu. Obdukcijom su ustanovljene učestale promene u traheji, vazdušnim kesama i plućima, a manifestovale su se kao: *tracheitis catarrhalis, aerosacculitis fibrinosa i pneumonia fibrinosa*.

U kontrolnim jatima označenim kao jato broj dva (jato 2) i jato broj tri (jato 3) u kojima je detektovano 4 % odnosno 8 % seropozitivnih pilića, nije bilo kliničkih znakova koji bi ukazivali na oboljenje, a proizvodni rezultati su daleko manje odstupali od tehnoloških normativa u poređenju sa jatom 1. Obdukcijom je utvrđeno da je broj jedinki sa promenjenim respiratornim organima u ova dva jata znatno manji u poređenju sa jatom 1. Pored toga, intenzitet promena na respiratornim organima u kontrolnim jatima je bio slabiji u poređenju sa jatom 1. U jatu 2 i jatu 3 nije zapažen ni jedan slučaj aerosakulitisa što je u jatu 1 bio učestao nalaz.