Aerosol Exposure of Ammonia, Dust and *Escherichia coli* in Broiler Chickens

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INTRODUCTION

Lancaster stated that in the condemnation of poultry carcasses in Canada, the important diseases were chronic respiratory disease (CRD), leucosis complex, tuberculosis, pericarditis, peritonitis and synovitis. Losses due to respiratory infection in chickens and the number of condemnations at federally inspected eviscerating plants in Canada as a result of respiratory diseases are second only to condemnation losses resulting from avian leucosis complex (14). With the recent introduction of turkey herpes virus vaccine, respiratory infections, mainly CRD and "air sac" disease syndrome, are now the primary cause of economic losses in the poultry industry (20).

The trend towards larger numbers of animals, automated feeding and handling, and confinement rearing in the livestock industry, has resulted in a highly artificial environment. This type of management practice has reached its climax in the poultry industry. However, little is known of the effects of environment on the susceptibility of birds to an infection, particularly the effect of high levels of ammonia and dust or the susceptibility of the respiratory tract to infection with *E. coli*.

Koon *et al*, evaluated the amount and type of dust resulting from broilers and laying hens housed in cages with and without litter (13). They observed that poultry dust was of two distinct types. In one type, the bulk of the matter was made up of flat, flaky and cellular materials ranging in diameter from one to 450 μm. These materials were identified as skin debris together with feed particles. The second type had long, cylindrical broken feather barbules of an average diameter of 4 μm and possessed nodes and internodes. Both types of dust particles had electrostatic charges which caused the formation of aggregates of various sizes. Dust production per bird raised in a cage with or without litter increased over the first six weeks of the growing period then levelled off, while birds raised on litter produced significantly more dust than those raised in cages without litter.

Attempts have been made to quantify ammonia (NH_3) in poultry houses under different environmental conditions. Scarborough and later Valentine studied the environment of different broiler houses; both observed NH_3 concentrations of the order of 60–70 ppm (18, 21). Anderson *et al* reported 50–100 ppm of NH_3 in some commercial poultry houses during the winter months and associated this high NH_3 concentration to reduced ventilation (3).

Anderson *et al*, demonstrated experimentally that exposure of chickens to 20 ppm NH_3 for 72 hours of 50 ppm NH_3 for 48 hours prior to exposure to an aerosol of Newcastle disease virus, significantly increased the susceptibility of the respiratory tract to the virus (2). Sato *et al*, demonstrated that the NH_3 remarkably enhanced the multiplication of *Mycoplasma gallisepticum* (Mg) in the respiratory tract (17).

Several authors incriminated poultry house dust as a reservoir of *E. coli* organisms. Carlson and Whenham recorded a total coliform count of about 360,000 per cubic foot (cu ft) of air when birds were about six weeks of age, after which the bacterial count started to drop until about nine weeks of age when the bacterial count per cu ft of air was 200,000 organisms. They observed a higher incidence of air sac disease among chickens, a week after the bacterial count peaked (4). Harry reported the coliform count per gm of dust in a depopulated broiler house to be between 200,000 and 800,000 with a preponderance of 0 serotypes *E. coli* (9, 10).

The objective of this project was to study the effect of NH_3, dust and *E. coli* either singly or in various combinations, on the normal respiratory tract of birds in an attempt to determine the possible role of these environmental factors in the development of CRD or air sac disease in broiler chickens.

MATERIALS AND METHODS

Experimental Design

Seven treatment groups, each consisting of 12 four week old chicks were exposed continuously for four weeks to NH_3, dust and *E. coli* either singly or in various combinations.

Temperature and relative humidity in each environmental chamber were controlled and uniform in all chambers. The environmental chambers used in the experiment were the same as those used and described by Doig (7). The intervals at which birds were euthanized for examination, the number of birds per group examined at

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TABLE I

TREATMENT GROUPS OF BIRDS EXPOSED TO AMMONIA, DUST AND *E. coli* AND POSTEXPOSURE SAMPLING TIMES

Group	Treatment	Time interval postexposure with no. of birds sacrificed						
		12 hr	24 hr	1 wk	2 wk	3 wk	4 wk	Total
I	NH_3	2[a]	2	2	2	2	2	12[a]
II	*E. coli*	2	2	2	2	2	2	12
III	Dust	2	2	2	2	2	2	12
IV	NH_3 and *E. coli*	2	2	2	2	2	2	12
V	NH_3 and Dust	2	2	2	2	2	2	12
VI	Dust and *E. coli*	2	2	2	2	2	2	12
VII	NH_3, Dust and *E. coli*	2	2	2	2	2	2	12
VIII	Control	2	2	2	2	2	2	12

[a]Number of birds examined

each sampling and the total number of birds in each treatment group are outlined in Table I.

Sterility of Inflowing Air

An Andersen sampler was used to test the sterility of the air in the environmental chamber (1). MacConkey agar plates were placed in the respective units of the air sampler, and 1 cu ft of air per minute was drawn for 30 minutes.

Source and Status of Experimental Birds

The birds used were obtained at one day of age from a commercial breeder[1] and hatched from *Mycoplasma gallisepticum* and *Mycoplasma synovia* free white leghorn crosses. Both sexes were equally represented.

Treatment

Ammonia was supplied as a 9.1% concentration by volume in compressed air, in 54.5 kg cylinders,[2] and released into the chamber through a pressure gauge, a valve and a flow meter. The concentration of NH_3 in the exposure chamber was measured twice daily using a sequential air sampler.[3] A standard volumetric method of analysis was used to determine the concentration of NH_3 in the chamber (11).

Dust was collected from the broiler units of a poultry farm, by using a brush to dust the floors around the feeders and roosts, the ceilings, ledges, walls, and equipment. It was collected in a large container, and screened in small amounts through a 0.08 cm wire mesh sieve and divided into convenient portions. Each portion of dust was spread evenly on tin foil and oven-dried at 200°C for 5 h. One g of the oven-dried dust was then cultured for bacteria and fungi. The dust was then weighed in 100 lots and stored in air-tight sterile polythene bags. The dust pumping apparatus consisted of a generator and a pressure pump. One hundred and fifty g of dust was put in the generator every day. After a 24 hour period, the amount of dust remaining in the generator was subtracted from 150, to give the amount of dust used per day. This value was divided by the internal volume of the chamber to obtain the concentration of dust per cu cm.

The strain of *E. coli* organism used in the experiment belonged to the serotypes 01 and 02, and was isolated from a field case of colisepticaemia in chickens.

Twenty aliquots of 800 ml of 3% trypticase soy broth (Difco), were prepared in litre sized conical flasks, autoclaved at 20 lb per sq in for 20 minutes, cooled and then inoculated with a 12 h *E. coli* broth culture from the original isolate. The inoculated media were incubated at 37°C for 24 hours and then distributed in 50 ml centrifuge tubes and centrifuged at 20,000 rpm at −15°C for 30 minutes. The supernatant was discarded and the sediments were resuspended in 20 ml of litmus skim milk, which is a preservative. A sterile 10 ml pipette was used to break up the bacterial clumps before pooling them in a Virtis freeze-drying tube and freezing them at −70°C. The Virtis tube was later connected to the on/off quickseal valve of a

[1]Shaver Poultry Breeding Farms Ltd., Cambridge, Ontario.

[2]Anhydrous Ammonia, Matheson of Canada Ltd., Whitby, Ontario.

[3]P.B. sequential sampler #2340-1, Research Appliance Co., Allison Park, Pennsylvania.

mobile freezer dryer and lyophilized for 48 hours. The lyophilized produce was stored in small sterile bottles in 5 g lots at −20°C.

The number of viable *E. coli* organisms present in 1 g of dust was determined using the bacteriological plate-count techniques (6, 15).

Lesions

(a) Gross. The respiratory tract of all birds were examined. The air sacs (both thoracic and abdominal) lesions were graded according to the following system:

- − absent
- \+ a few cloudy flecks and thickening
- ++ extensive clouding and thickening and possibly accompanied by serous or thin frothy exudate
- +++ extensive clouding and thickening accompanied by thick mucoid to mucupurulent exudate but not caseous
- ++++ extensive clouding and thickening, accompanied by fibrinopurulent exudate (caseous).

(b) Microscopic. The epithelium of each of the turbinate, trachea and air sacs was examined for deciliation, surface exudate, metaplasia, necrosis and changes in number and size of the goblet cells. The lamina propria of the same tissues was observed and scored for presence of congestion, edema, hemorrhage, inflammatory cellular infiltration, proliferation of lymphoid follicles (bursal dependent nodes or BDN), necrosis and mucous gland thickness. The lungs were examined for the presence of congestion, hemorrhage, edema alveolar exudate, alveolar macrophages, inflammatory cellular infiltrate, bursal dependent nodes, necrosis and granulomatous reaction.

Each of the histological lesions noted above were classified according to the degree of involvement as follows:

1. mild degree of involvement
2. intermediate degree of involvement
3. severe involvement
4. very severe involvement

The microscopic lesion scores per tissue per bird were then added up for each treatment group to obtain the group total tissue score. This value was divided by the number of birds in the group to arrive at the mean lesion score per tissue per group. The group total lesion scores recorded was the sum of each total tissue score in a treatment group.

The turbinate, upper, middle and lower portions of the trachea, lungs and thoracic and abdominal air sacs were collected and fixed in 10% buffered formalin solution, embedded in paraffin, sectioned at 5 μm and stained with hematoxylin and eosin.

RESULTS

The NH_3 concentration in the chambers varied from 100.65 to 100.98 ppm. the amount of dust pumped into the chambers varied from 93.50 to 95.94 g per day. The average number of *E. coli* organisms contained in 1 g mixture of dust and *E. coli* was 33×10^4.

The mean dust concentration in the chamber varied from 101 mg/cm^3 to 103.72 mg/cm^3 and proved to be sterile on examination.

Throughout the four week duration of the ex-

TABLE II

GROSS LESIONS OF AIR SACS IN THE BIRDS EXPOSED TO AMMONIA, DUST AND *E. coli* RECORDED DURING SEQUENTIAL EXAMINATION OF CHICKENS

		Thoracic Air Sac						Abdominal Air Sac					
Group	Treatment	12 hr	24 hr	1 wk	2 wk	3 wk	4 wk	12 hr	24 hr	1 wk	2 wk	3 wk	4 wk
I	NH_3	−	+	+	−	−	−	−	−	−	−	−	−
II	*E. coli*	−	−	−	−	−	−	−	−	−	−	+	−
III	Dust	−	−	+	−	+	−	−	−	−	−	+	−
IV	NH_3 and *E. coli*	−	+	−	++	−	+	−	−	−	−	++	+
V	NH_3 and Dust	−	−	+	−	+	−	−	−	−	−	+	−
VI	Dust and *E. coli*	−	+	+	++	++	+	−	−	−	−	−	+
VII	NH_3, Dust and *E. coli*	−	+	++	+++	−	+	−	+	+	−	++	+
VIII	Control	−	−	−	−	+	−	−	−	−	−	−	−

TABLE III

RESULTS OF THE HISTOLOGICAL EVALUATION OF LESIONS IN THE BIRDS EXPOSED TO AMMONIA, DUST AND *E. coli* AND CONTROLS

		Tissue and Lesion Score											
		Turbinate		Trachea		Lung		TAS[c]		AAS[d]			
Group	Treatment	T[a]	M[b]	T	M	T	M	T	M	T	M	GTLS[e]	Rank
I	NH_3	2	0.17	12	1.00	22	1.83	7	0.64	0	0	43	3
II	*E. coli*	0	0	4	0.36	4	0.36	5	0.45	3	0.27	16	2
III	Dust	0	0	8	0.67	24	2.00	12	1.00	6	0.5	50	4
IV	NH_3 and *E. coli*	0	0	22	1.83	28	2.33	20	1.67	10	0.83	80	7
V	NH_3 and Dust	4	0.36	14	1.17	30	2.50	18	1.50	8	0.67	74	5
VI	Dust and *E. coli*	0	0	18	1.50	35	2.92	14	1.17	12	1.00	79	6
VII	NH_3, Dust and *E. coli*	8	0.73	28	2.33	40	3.33	30	3.00	15	1.50	121	8
VIII	Control	0	0	0	0	2	0.17	0	0	1	0.08	3	1

[a]T = Total lesion score/Tissue/Group
[b]M = Mean lesion score/Tissue/Group
[c]TAS = Thoracic air sac
[d]AAS = Abdominal air sac
[e]GTLS = Group total lesion score

periment, there were no deaths in any of the treatment groups or the controls.

Following gassing with NH_3, respiratory depression was observed in birds of Groups I, V and VII. The first response which occurred a few minutes after the flow rate meter was turned on and the pressure gauge was released, was head shaking, rubbing of the eyes under the wing feathers, and swift movement within the chamber, probably seeking a more accommodating environment. Later, there was occasional gasping for breath, and sometimes birds would stumble against the feed and water trough, or lie beside the plexiglass door. Although this malaise lasted for only a brief period, it repeated itself each time the NH_3 was shut down for two hours at cleaning period, and then turned on.

The NH_3 exposed birds also consumed less food and weighed less than birds in other chambers as judged by the amount of left over feed and the light weight of these birds at cleaning and sampling intervals, coupled with the slight to marked emaciation of these birds at necropsy.

In the dust only treatment, the birds did not exhibit any abnormal clinical signs.

Gross lesions in the lungs in all groups were minimal with the exception that at one week post exposure the lungs in birds of group VII were markedly congested and contained yellowish necrotic foci (Table II).

The microscopic lesions are recorded in Table III. In order to evaluate and interpret the microscopic findings, the treatment groups were assessed on the basis of lesion scores, using the Friedman two-way nonparametric analysis; $P < 0.05$ was regarded as significant (8, 11). As evidenced in Table III the more severe lesions were produced in birds of Group VII. Focal squamous metaplasia of the tracheal epithelium (Figure 1), and hypertrophy of the mucosa (Figure 2) were observed in this group. The lungs of birds in Group IV frequently revealed granulomatous lesions (Figure 3). The air sacs in birds of Group VII were almost universally involved and showed coagulation necrosis and a fibrinocellular surface exudate (Figure 4).

DISCUSSION

Exposure of chickens to either NH_3 or dust results in mild to moderate macroscopic and microscopic changes in the trachea, lungs and particularly in the air sacs. Birds exposed to *E. coli* alone are relatively unaffected, but when exposed to a combination of *E. coli* and either NH_3 or dust, the birds manifest marked pathological lesions. This confirms the nonpathogenic nature of *E. coli* organism for an intact respiratory tract, and also demonstrates the devitalizing effect of NH_3 and dust on the respiratory mucosa.

Ammonia or dust cause deciliation of the epithelium of the upper portion of the trachea, probably restricting the effectiveness of the mechanical defense mechanism of the respiratory

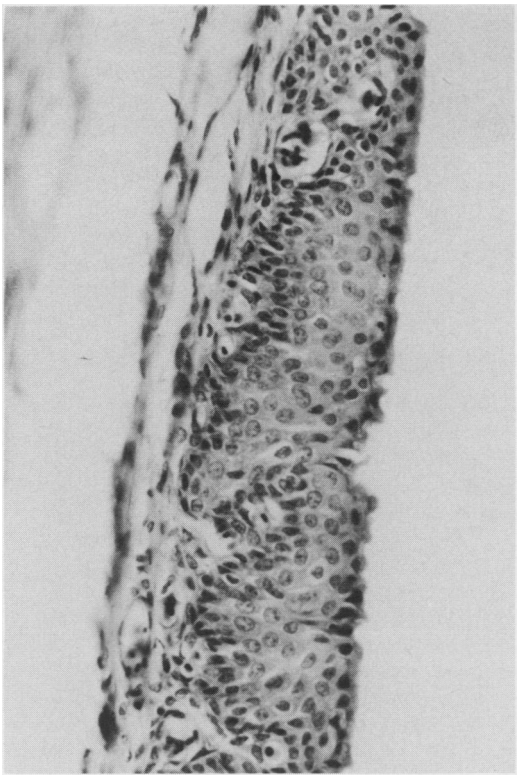

FIGURE 1. Trachea two weeks postexposure. Focal squamous metaplasia of the tracheal epithelium in Group VII birds exposed to NH_3, dust and *E. coli* (H & E).

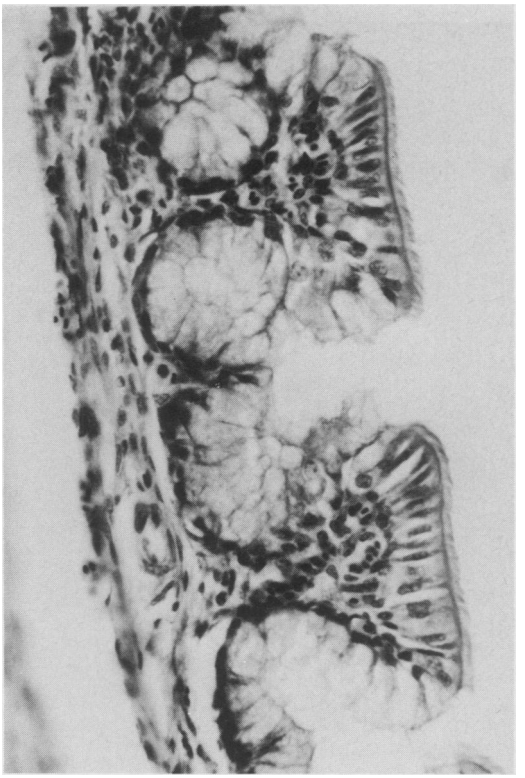

FIGURE 2. Trachea two weeks postexposure. Hypertrophy of the tracheal mucous glands in Group VII birds exposed to NH_3, dust and *E. coli* (H & E).

system. This damage is further perpetuated by an increased mucous secretion which could promote the multiplication of *E. coli* organism (17), and by reversed ciliary waves or by other mechanisms, the organism could gain access to the lungs and the air sacs. In the lungs, it stimulates an acute inflammatory response, characterized by vacular congestion, edema, heterophil and mononuclear cell infiltration. In the subacute reaction, the significant lesions consist of lymphoid cell hyperplasia and macrophage infiltration. A chronic granulomatous reaction is observed in the lungs of Group VII birds, a week postexposure. The acute reaction in the air sacs is marked by edema, heterophil infiltration and later by macrophage and small mononuclear cellular infiltration. After a prolonged irritation, there is goblet cell hyperplasia along the air sac epithelium.

Lesions occur more frequently in the thoracic air sacs than the abdominal air sacs, during the first two weeks of exposure. This is likely due to an increased resident time of the contaminated air in the thoracic sacs (19).

The reduction in feed consumption observed in the birds housed in exposure chambers containing ammonia has been reported previously (5, 16). A reduced respiratory rate was seen and on this basis, it was assumed that the reduction in feed consumption is a result of a lower energy requirement since respiratory evaporation contributes to heat loss in birds (5).

The atmospheric levels of ammonia concentration used in these experiments are at the upper levels reported as occurring in commercial broiler houses (3). These levels were used in order to attain pathogenic manifestations in the respiratory tracts of the birds, within the time limits set for the experiments.

It was necessary to stop the gas flow from time to time to clean the chambers. When the gas was turned to again clinical signs of respiratory distress were seen, but these signs were transitory and the birds appeared to adapt to the NH_3 concentration, without clinical signs.

The coliform counts in the dust in chambers approximate the levels that have been reported in the dust of commercial broiler houses (9).

Air sac infection can be caused by several infectious agents including Newcastle disease virus, infectious bronchitis virus, infectious laryngotracheitis virus, *E. coli* and *Mycoplasma gallisepticum*. From a diagnostic standpoint, however, environmental factors as demonstrated in this experiment, can play as important a role in the development of air sac infection as the infec-

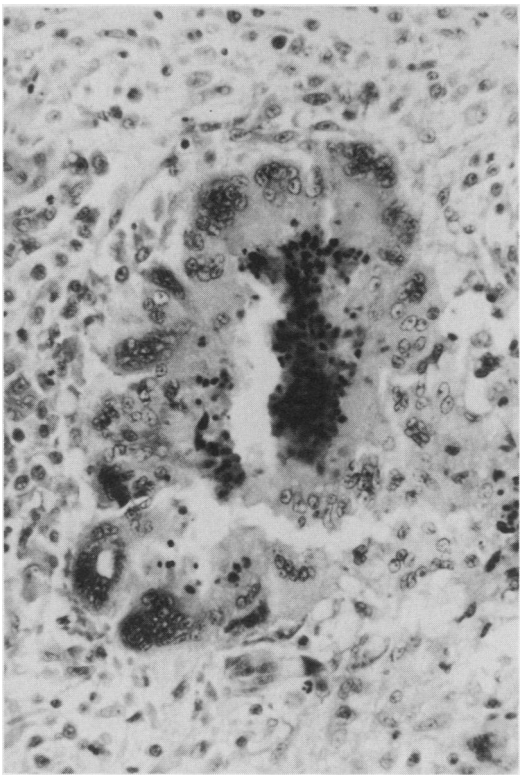

FIGURE 3. Lung one week postexposure. Granulomatous lesion with giant cells and macrophages surrounding a necrotic focus in Group VII birds exposed to NH_3, dust and *E. coli* (H & E).

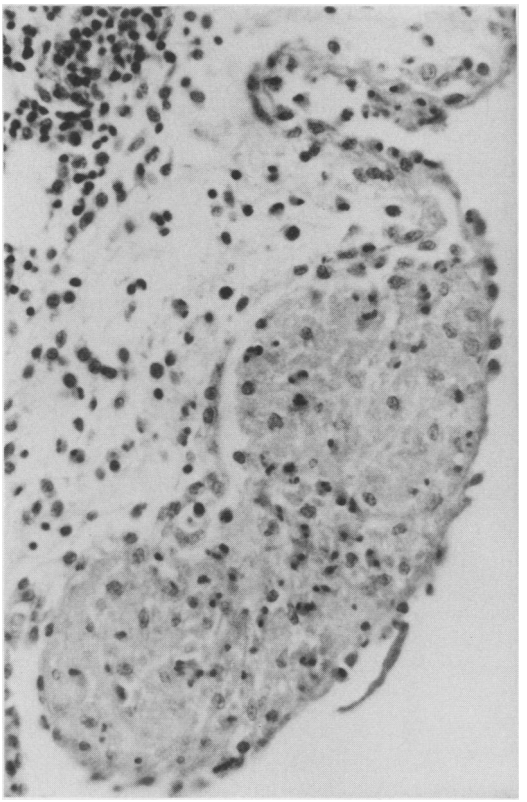

FIGURE 4. Thoracic air sac one week postexposure. Coagulation necrosis of the surface epithelium and fibrino-cellular surface exudate. The lamina propria was edematous and infiltrated with heterophils and mononuclear cells in Group VII birds exposed to NH_3, dust and *E. coli* (H & E).

tious agents. Moreover, it would appear that air sac infection caused by *E. coli* which is responsible for a great percentage of the condemnation loss in the broiler industry, could be controlled by stringent management of the birds' environment. Improving the environmental conditions with particular reference to ventilation rates, relative humidity and temperature would help to cut down on the level of dust and NH_3 in a broiler house, and thus reduce the incidence of air sac infection. The results of these studies confirm the findings of other workers that devitalization of the air sacs by physical factors greatly enhances the invasion and multiplication of infectious agents in the air sacs (2, 17).

SUMMARY

The effects of exposing the normal respiratory tract of chickens to ammonia (NH_3), dust and *Escherichia coli* were investigated. Four week old mycoplasma free broiler chickens were exposed in environmentally controlled chambers to aerosols of NH_3, dust and *E. coli* in various combinations. Each group consisted of 12 birds and another group of 12 four week old birds which received none of the factors served as the control. The exposure was continuous for four weeks and at 12 hour and 24 hours postexposure and subsequently at weekly intervals, two birds from each treatment group were sacrificed randomly and examined for lesions.

Ammonia caused some transient discomfort to the chickens, resulting in reduced food consumption and weight loss. Gross and microscopic lesions on air sacs were observed as early as 12 hours postexposure in birds exposed to NH_3 alone or in combination with either *E. coli*, dust or both. The combination of all three factors gave the most severe reactions. A week postexposure, the chickens exposed to all three factors also developed granulomatous lesions in the lungs.

RÉSUMÉ

Cette expérience visait à étudier les effets de l'ammoniac (NH_3), de la poussière et d'*Escherichia coli* sur la muqueuse respiratoire de poulets sains. On plaça donc des poulets de gril, âgés de quatre semaines et exempts de mycoplasmes, dans des pièces à atmosphère contrôlée; on les soumit ensuite à des vaporisations de di-

vers mélanges des trois éléments suivants: NH_3, poussière et *E. coli*. Chacun des sept groupes expérimentaux comptait 12 poulets; le groupe témoin se composait aussi de 12 poulets. On vaporisa de façon continuelle et durant quatre semaines. Au bout de 12 et 24 heures après la fin de l'expérience, ainsi qu'à intervalles ultérieurs hebdomadaires, on sacrifia au hasard deux des sujets de chacun des groupes expérimentaux et on procéda à la recherche de lésions.

L'ammoniac provoqua un malaise passager qui se traduisit par de l'anorexie et une perte de poids. Dès 12 heures après la fin de l'expérience, on décela des lésions macroscopiques et histologiques dans les sacs aériens des poulets soumis à des vaporisations de NH_3, seul ou mélangé avec de la poussière et/ou *E. coli*. La vaporisation simultanée de ces trois éléments provoqua les lésions les plus graves. Une semaine après la fin de l'expérience, les poulets soumis à la vaporisation de trois éléments présentaient aussi des lésions pulmonaires granulomateuses.

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