RESPIRATORY DISEASES OF CHICKENS AND TURKEYS

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Cover

This broiler chick is exhibiting dyspnea because of aspergillosis, the most common mycosis of young poultry. Chicks become infected by inhaling airborne fungal spores from either the hatchery or the poultry house environment. Aspergillosis can be prevented through proper management.
[Cover photo by Fred Hoerr; cover design by Steve Winslept]

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Program

Anatomy and Physiology of the Avian Respiratory System

M. R. Fedde

The respiratory system is one vital part of the pathway for oxygen and carbon dioxide transfer between the atmosphere and mitochondria of body cells. Its fundamental function is the transfer of these gases between the atmosphere and the blood. The different classes of vertebrates have evolved considerably different techniques to accomplish this task and all birds so far studied seem to have many common features of their respiratory system. It is the purpose of this brief communication to point out those common features that are of most practical interest to investigators studying the various respiratory diseases of poultry.

Increases in our understanding of various organ systems seem to occur in spurts. Such has been the case with the avian respiratory system. During the 1960's and 1970's, there were many investigators around the world actively studying this system. The results of their studies and interaction resulted in a rapid increase in knowledge of almost every facet of both structure and function. More recently, however, funding for these studies has waned and most investigators have been forced to shift their emphasis to other areas. Although much has been learned about the system, we still do not know details of the system's function during the most fundamental activities of a birds life, ie., flying and diving. Questions about how some birds fly at altitudes of 9,000 meters (29,530 feet) or dive to depths of 265 meters (870 feet) remain to be answered. Understanding the involvement of the respiratory system during natural behaviors will undoubtedly aid in answering many practical problems faced by poultry producers.

STRUCTURAL PRINCIPALS OF THE AVIAN RESPIRATORY SYSTEM

The extrapulmonary tubular system consists of the upper airways in the head, the larynx, the trachea, the syrinx, and the extrapulmonary primary bronchus (6,7,8,10,14). This part of the respiratory system conducts gases from the atmosphere to the lungs and functions to heat, humidify and filter the inspired gas. It also functions in vocalization. The trachea varies greatly in length and may be very long and coiled in some birds (much longer than the total length of the bird in some species, such the trumpet bird or the whooping crane) (4,11,15,18,26). Thus, there is sometimes a large dead space volume that may contribute to a large tidal volume and low respiratory frequency (13).

The lungs function as organs of gas exchange. They have a low compliance (do not expand or contract much during breathing) and lie in the dorsal-lateral region of the thorax, extending between the ribs. They contain only a small gas volume. There are only three hierarchies of bronchi in each avian lung: 1) a single intrapulmonary primary bronchus that is the continuation of the extrapulmonary primary bronchus and courses in an S-shape from the medioventral surface of the lung to the dorsolateral surface; 2) three major sets of secondary bronchi (medioventral secondary bronchi that leave the intrapulmonary primary bronchus shortly after its origin and course over the medioventral surface of the lung, mediodorsal secondary bronchi that arise from the intrapulmonary primary bronchus after it has passed to the dorsolateral surface of the lung and course over that surface of the lung just beneath the ribs, and the lateroventral secondary bronchi that leave the intrapulmonary primary bronchus just opposite the origin of the mediodorsal secondary bronchi and course over the lateroventral surface of the lung); and 3) tertiary bronchi (parabronchi) in whose walls gas exchange with the blood occurs. There are two sets of parabronchi in most birds: 1) paleopulmonic parabronchi that connect mediodorsal to medioventral secondary bronchi and make up 80% or more of the gas exchange surface; and 2) neopulmonic parabronchi that connect mediodorsal

and lateroventral secondary bronchi to the caudal set of air sacs and make up 20% or less of the gas exchanging surfaces of the lung. Neopulmonic parabronchi are not present in penguins or emus.

The air sacs in birds arise from the various secondary bronchi and the continuation of the primary bronchus. The cranial set (clavicular, cervical and cranial thoracic) arises from the medioventral secondary bronchi. The caudal set (caudal thoracic and abdominal) arises from the lateroventral and mediodorsal secondary bronchi and the continuation of the intrapulmonary primary bronchus. There are nine air sacs in most birds: An unpaired clavicular, and paired cervical, cranial thoracic, caudal thoracic, and abdominal air sacs. These sacs occupy all available space in the thoracoabdominal cavity not occupied by other organs. They are extremely compliant and poorly vascularized. There is essentially no gas exchange with the blood in their walls. They are often destroyed during necropsy of birds unless extreme care is used.

VENTILATION OF THE AVIAN RESPIRATORY SYSTEM

When a bird breathes, the entire thoracoabdominal cavity changes volume. The sternum, furcula and coracoid pivots about the shoulder (22,27). This motion also moves the ribs laterally so the body volume increases in both a dorsoventral and a lateral direction. These movements result from contraction of the trunk muscles. Muscles active during inspiration include M. scalenus, M. costosternalis pars major, M. intercostales externi, and Mm. levatores costarum (5,9). Muscles active during expiration include M. costosternalis pars minor, Mm. intercostales interni, M. obliquus externus abdominis, M. obliquus internus abdominis, M. rectus abdominis, and M. transversus abdominis. Both inspiration and expiration require contraction of the various trunk muscles, even in quiet breathing. When the trunk muscles are not contracting, the sternum is approximately in its midposition between the peak of inspiration and the peak of expiration. The pectoralis and supracoracoideus muscles do not appear to be involved in respiration, even during flight.

The pressure changes in the air sacs, relative to the atmosphere, result from changes in the volume of the thoracoabdominal cavity when the trunk muscles alternately contract and relax. During inspiration, the volume of the air sacs increases, the pressure inside them decreases below that in the atmosphere, and gases enter the respiratory system. During expiration, the volume of the air sacs decreases, the pressure increases above that in the atmosphere, and gases leave the respiratory system. The resulting pattern of gas flow through the lung may be directly related to primary sites of infection. During inspiration, part of the inspired gas flows into the mediodorsal secondary bronchi and into the paleopulmonic parabronchi to the cranial air sacs; the other part of the inspired gas flows either through neopulmonic parabronchi and into the caudal air sacs, or directly into the caudal air sacs (2,22). Thus, in the latter case, contaminated gas can reach the caudal air sacs without contacting the epithelial surfaces of the parabronchi. During expiration, gas leaves the cranial air sacs and flows through medioventral secondary bronchi, out through the trachea to the atmosphere. At this time, gas leaves the caudal air sacs and flows through the neopulmonic parabronchi into the mediodorsal secondary bronchi and paleopulmonic parabronchi. Thus, gas flows unidirectionally through the paleopulmonic parabronchi during both inspiration and expiration but bidirectionally through the neopulmonic parabronchi.

The above pattern of gas flow results from aerodynamic valving within the lung (1,3,24). This valving results from forces due to gas stream motion and can not be attributed to any defined anatomical valves in the bronchial system. The aerodynamic valving involves the gas flow rate through the primary bronchus, gas density, and geometry of the primary bronchus upstream from the origin of the medioventral secondary bronchi.

The net result of the unique pattern of gas flow through the lung is a high partial pressure of CO_2 and low partial pressure of O_2 in the gas in the cranial air sacs (near that in expired gas) and a much lower partial pressure of CO_2 and higher partial pressure of O_2 in the gas in the caudal air sacs (modified from inspired gas partial pressures by gas exchange in the neopulmonic parabronchi and reinhaled dead space gas) (21).

GAS EXCHANGE IN THE AVIAN LUNG

Gases are exchanged with the blood in the wall of the parabronchi (17,23). Invaginations in the parabronchial wall (atria and infundibuli) lead to air capillaries (3 to 10 μm in diameter in various species) which present a large surface area and a very thin barrier for gas exchange (16). Oxygen moves from the convective gas stream of the parabronchial lumen by diffusion into the air capillaries, across the thin blood-gas barrier, and into the blood. Carbon dioxide moves by diffusion in the opposite direction. Blood capillaries radiate from the interparabronchial and intraparabronchial arteries into the parabronchial wall to intertwine with the air capillaries. The mixed venous blood thus enters the gas exchange area at approximately a 90 degree angle with respect to the convective gas flow in the parabronchial lumen. This forms an anatomical arrangement called a cross-current gas exchange system. The partial pressure of oxygen in the blood leaving this system has the potential to be higher than its partial pressure in the gas leaving the parabronchus. Thus, this type of system can have a high efficiency for gas exchange.

CONTROL OF BREATHING

The avian respiratory control system is responsible for keeping ventilation in concert with the metabolic rate during varied activity states (12). This system has five basic components: Receptors, afferent neural pathways, central coordinating neurons, efferent neural pathways, and effector organs. Unlike mammals, birds have intrapulmonary chemoreceptors that generate an increase in afferent impulses when carbon dioxide partial pressure in the intrapulmonary gas is low. These receptors seem to be important in determining the pattern of breathing. Birds also possess chemoreceptors in the carotid bodies and in the brain. The central integrating neurons are in the pons and medulla oblongata, and they respond to neural input from higher brain regions as well as from peripheral chemoreceptors. Exercise often causes hyperventilation (increase in ventilation relative to the metabolic production of carbon dioxide so that the arterial partial pressure of carbon dioxide decreases), indicating the presence of a feed-forward component in the control system. The neural output from the control centers modify the strength and frequency of contraction of the respiratory muscles, resulting in changes in the volume of the thoracoabdominal cavity and associated changes in ventilation.

CLINICAL APPLICATIONS

Improved understanding of the structure and function of the avian respiratory system has allowed improvement in many medical and management practices.

1. Unidirectional ventilation techniques can be used with birds because the lung is a "flow-thru" lung rather than a "flow-into" lung. This can be useful for induction of anesthesia (25).
2. Air sacs can be cannulated so that spontaneous breathing can continue during tracheal obstruction (19,20).

3. Endoscopic examination of the interior of the air sacs can be done when infectious agents are suspected to be colonizing these structures.

4. Intraperitoneal injections of drugs must be done with great care so the drug is not placed in an air sac.

5. Opening of air sacs during thoracic or abdominal surgery may seriously impair ventilation of the lungs.

6. When restraining birds, the thoracic and abdominal motion must not be hindered or ventilation will be reduced. Placement in the supine position may also cause respiratory embarrassment.

7. Manual artificial ventilation can be done by gently compressing the sternum and allowing it to recoil to its resting position.

REFERENCES

1. Banzett, R. B., J. P. Butler, C. S. Nations, G. M. Barnas, J. L. Lehr, and J. Jones. Inspiratory aerodynamic valving in goose lungs depends on gas density and velocity. Respir. Physiol. 70:287-300. 1987.

2. Brackenbury, J. H. Airflow and respired gases within the lung-air-sac system of birds. Comp. Biochem. Physiol. 68A:1-8. 1981.

3. Butler, J. P., R. B. Banzett, and J. J. Fredberg. Inspiratory valving in avian bronchi: Aerodynamic considerations. Respir. Physiol. 72:241-156. 1988.

4. Clench, M. H. Tracheal elongation in birds-of-paradise. Condor. 80:423-430. 1978.

5. DeWet, P. D., M. R. Fedde, and R. L. Kitchell. Innervation of the respiratory muscles of Gallus domesticus. J. Morphology. 123:17-34. 1967.

6. Duncker, H-R. Structure of the avian respiratory tract. Respir. Physiol. 22:1-19. 1974.

7. Duncker, H-R. Structure of avian lungs. Respir. Physiol. 14:44-63. 1972.

8. Duncker, H-R. The lung air sac system of birds. A contribution to the functional anatomy of the respiratory apparatus. Engeb. Anat. Entwicklungsgesch. 45(6):1-171. 1971.

9. Fedde, M. R. Respiratory muscles. In: Bird respiration, vol. I. T.J. Seller, ed. CRC Press, Boca Raton, FL. pp. 3-37. 1987.

10. Fedde, M. R. Respiration. In: Avian physiology, 4th ed. P.D. Sturkie, ed., Springer Verlag, New York. pp. 191-220. 1986.

11. Gaunt, A. S., S. L. L. Gaunt, and H. D. Prange. Acoustic properties of coiled trachea in Gruidae. Am. Zool. 23:895. 1983.

12. Gleason, M., and V. Molony. Control of breathing. In: Form and function in birds, vol. 4. A.S. King and J. McLelland, eds. Academic Press, New York. pp. 439-484. 1989.

13. Hinds, D. S., and W. A. Calder. Tracheal dead space in the respiration of birds. Evolution. 25:429-440. 1971.

14. King, A. S. Structural and functional aspects of the avian lungs and air sacs. In: International review of general and experimental zoology, vol. 2. W.J.L. Felts and A.S. King, eds. Academic Press, New York. pp. 171-267. 1966.

15. Latham, J., and L. S. Romsey. An essay on the trachea or windpipes of various kinds of birds. Trans. Linnean Soc. Lond. 4:90-128. 1798.

16. Maina, J. N. The morphometry of the avian lung. In: Form and function in birds, vol. 4. A.S. King and J. McLelland, eds. Academic Press, New York. pp. 307-368. 1989.

17. Powell, F. L., and P. Scheid. Physiology of gas exchange in the avian respiratory system. In: Form and function in birds, vol. 4. A.S. King and J. McLelland, eds. Academic Press, New York. pp. 393-437. 1989.

18. Prange, H. D., J. S. Wasser, A. S. Gaunt, and S. L. L. Gaunt. Respiratory responses to acute heat stress in cranes (Gruidae): The effects of tracheal coiling. Respir. Physiol. 62:95-103. 1985.

19. Rode, J. A., S. Bartholow, and J. W. Ludders. Ventilation through an air sac cannula during tracheal obstruction in ducks. J. Assn. Avian Vet. 4:98-102. 1990.

20. Rosskopf, W. J., and R. W. Woerpel. Abdominal air sac breathing tube placement in psittacine birds and raptors its use as an emergency airway in cases of tracheal obstruction. Proc. Assoc. Avian Vet. 215-217. 1990.

21. Scheid, P., M. R. Fedde, and J. Piiper. Gas exchange and air-sac composition in the unanesthetized, spontaneously breathing goose. J. Exp. Biol. 142:373-385. 1989.

22. Scheid, P., and J. Piiper. Respiratory mechanics and air flow in birds. In: Form and function in birds, vol. 4. A.S. King and J. McLelland, eds. Academic Press, New York. pp. 369-391. 1989.

23. Scheid, P. and J. Piiper. Gas exchange and transport. In: Bird respiration, vol. 1. T.J. Seller, ed. CRC Press, Boca Raton. pp. 97-129. 1987.

24. Wang, W., R. B. Banzett, J. P. Butler, and J. J. Fredberg. Bird lung models show that convective inertia effects inspiratory aerodynamic valving. Respir. Physiol. 73:111-124. 1988.

25. Whittow, G. C., and N. Ossorio. A new technique for anesthetizing birds. Lab. Anim. Care. 20:651-656. 1970.

26. Yarrell, W. Observations on the trachea of birds; with descriptions and representations of several not hitherto figured. Trans. Linnean Soc. London. 15:378-391. 1827.

27. Zimmer, K. Beiträge zur Mechanik der Atmung bei den Vogeln in Stand und Flug. Zoologica. 33 (5Heft 88):1-69. 1935.

Avian Respiratory Defense Mechanisms, Injury, and Repair

Martin D. Ficken

The respiratory membrane is the most extensive of all tissues that interfaces directly between animals and their environment. Protection of this interface is accomplished by several nonimmune-mediated pulmonary defense mechanisms which include aerodynamic filtration, mucociliary transport in the nasal cavity and tracheobronchial tree, entrapment in the trilaminar substance of the atria and infundibula of the lung, and phagocytosis and removal by phagocytic cells (18). These nonspecific defenses are amplified and augmented by specific immune responses integrated into the pulmonary defense system. A vast body of knowledge is available on pulmonary defense of mammalian systems; however, very little is known about these activities in the bird. Much of what is presented in the following discussion is a mixture of relevant research of the subject in avian systems combined with what can be extrapolated from what is known in mammals.

RESPIRATORY DEFENSES

Pulmonary clearance mechanisms are critical to nonspecific pulmonary defense and can be subdivided into two broad categories, particle clearance and bacterial clearance (18). Particle clearance is defined as the physical removal of inhaled particles from the respiratory tract over a period of time following their initial deposition. Bacterial clearance, on the other hand, refers to the killing or removal of bacteria during a period of time following the initial deposition of bacteria in the respiratory tract.

Particle Clearance. Particle clearance depends upon the site and amount of initial particle deposition. Deposition refers to the initial processes that determine what fraction of the particles in inspired air are caught in the respiratory tract and fail to exit with expired air (5). Factors influencing the deposition of an aerosol are the size of the aerosolized particles which can be affected by their hygroscopicity, the electric charge of the particles, breathing pattern, species differences, and respiratory tract disease. The most important of these factors is particle size. In birds, large particles (3.7- to 7 microns in diameter) are deposited in the nasal cavity and anterior trachea, whereas smaller particles (0.091- to 1.1 microns) are deposited uniformly throughout the remainder of the respiratory tract (20). Viruses, many bacteria, fungal spores, and dust particles are small enough to be distributed into the lower respiratory tract of birds during normal respiration which is the critical area relating to serious disease.

Particle clearance is accomplished through a combination of mucociliary transport by the tracheobronchial tree, entrapment in the trilaminar substance of the atria and infundibula of the lung, and phagocytosis and removal by phagocytic cells. Particles deposited on the ciliated epithelium of the tracheobronchial tree are rapidly removed via mucociliary transport (23). In the lung, particles which bypass the tracheobronchial tree often lodge on the trilaminar substance lining the atria and infundibula (31). This substance is secreted by the epithelial cells which line the atria and infundibula and is unique to the avian lung. These same cells also phagocytize inhaled particles and then discharge them through the basement membrane to the interstitium where they are picked up by resident macrophages (31). What happens to the particles after they are ingested by these macrophages is unknown, but they may be degraded and stored in these cells or the material may be transported to the blood or lymph. The bird is different from mammalian counterparts since it does not have any "resident" phagocytic cells within the air capillaries (31) or the blood vasculature ("intravascular macrophages") (22) for clearance of particles. Air sacs have "ciliated islands" (16) scattered over their surface which,

although their function is unknown, may remove particles by mucociliary transport during expiration when the air sacs are collapsed. Like the lung, the air sac has no resident population of macrophages either on the membrane surface or within the interstitium (12,33).

Bacterial clearance. Bacterial clearance, as opposed to particle clearance, is accomplished by nonspecific mechanisms such as phagocytosis and killing by phagocytic cells, physical removal by mucociliary transport, and lysis mediated by complement fixation (18). In the trachea, physical removal by mucociliary transport is the most important mechanism of bacterial clearance. In the lung, mucociliary transport mechanisms are undoubtedly important; however, bactericidal mechanisms are thought to be more important in the airways distal to the bronchus-parabronchus junction, i.e. areas not covered by a ciliated epithelium. These distal regions are most likely protected by the trilaminar substance lining the atria and infundibula, the atrial and infundibular epithelial cells, and the interstitial macrophages (31). The trilaminar substance is thought to contain substances which are bactericidal, although no experimental evidence is yet known, similar to antibacterial factors found in alveolar lining material of mammals (6,7,21). As described above, the atrial and infundibular epithelial cells and interstitial macrophages are phagocytic and are postulated, but not proven, to be capable of bacterial killing. During bacterial challenge of the lung there is also an influx of heterophils, macrophages, proteins, and complement components from the blood which are capable of phagocytosis and bactericidal activity (9,26,35). Like the lung, noncellular substances line the air sacs. It is unknown if this material has bactericidal activity or if the epithelial cells of the air sac are phagocytic similar to what is postulated for the lung. It is known, however, that an influx of heterophils, macrophages, and proteins occurs in the air sac and is partially responsible for bacterial clearance (10,11,34,35).

Mucociliary Transport. Proper functioning of the defense mechanisms of the upper and lower respiratory tract depends on the integrity of the lining membrane. Any deficiency of, or insult to, this membrane leads to inhibition of pulmonary clearance of particulates and bacteria. The end result is at best, an "inefficient" bird due to inadequate oxygen:carbon dioxide exchange in a damaged lung and at worst, overwhelming sepsis leading to death. As described above, mucociliary clearance by ciliated cells is critical for removal of the large volumes of particulates inhaled each day and inhibition of this activity increases the numbers of particulates (including infectious organisms) and the time these particulates are in contact with the respiratory membrane. Inhibition of mucociliary clearance can occur by ciliostasis (nonfunctional cilia), deciliation (loss of cilia) of epithelial cells, or death of the ciliated epithelial cells (18). Ammonia causes ciliostasis and deciliation of epithelial cells (24,25). Bordetella avium attaches to the cilia of the epithelial cells and leads to deciliation and loss of ciliated cells (3,14). Respiratory viruses (i.e. Newcastle disease virus, Yucaipa virus, influenza viruses, and adenoviruses) replicate in and kill epithelial cells leading to loss of mucociliary transport (1,15). Excess dust leads to increased mucus secretion which is difficult to clear and also allows increased contact between inhaled particulates and the respiratory membrane.

Phagocytosis and Bacterial Killing. Phagocytosis and killing of bacteria by phagocytic cells, a second major mechanism of pulmonary clearance, can also be inhibited by a variety of insults. Excess dust is phagocytized by macrophages and can inhibit the bird's ability to phagocytize other particulates, particularly bacteria. This may lead to chronic low-grade inflammation of the respiratory tract and allow colonization by the bacteria. Ammonia, in addition to effects on ciliated cells, can cause failure of bacterial killing by macrophages (17). Newcastle disease virus (and other viruses) like ammonia, causes a decrease in phagocytic activity and bacterial killing by influxing phagocytes although by a different mechanism (13). Influenza virus replicates in and kills the resident phagocytic cells of the avian lung, the atrial and infundibular epithelial cells (32).

Noncellular Lining Substance. The third major (postulated) mechanism of bacterial clearance of the avian lower respiratory tract, the noncellular material lining the atria, infundibula, air capillaries, and air sacs, may be inhibited if birds are deprived of essential fatty acids. In mammals, material lining the air spaces of the lower respiratory tract is composed of surfactant, lipids, and protein. Recent studies have indicated that this material has bactericidal activity and this is of primary importance in the defense of the lungs against infection (6,7,21). The antibacterial activity of this material is confined to the free fatty acid fraction. In chickens fed essential fatty acid deficient diets, a respiratory disease syndrome develops which can not be attributed to any particular pathogen, but rather to essential fatty acid deficiency and whatever role these factors play in respiratory defense (8). In addition, the essential fatty acid deficient chicken, which is a model for cystic fibrosis, shows that pulmonary lesions can be modified depending upon the type of dietary oils consumed (8).

RESPIRATORY INJURY AND REPAIR

Once damage occurs in the respiratory tract, bacteria adhere to and colonize damaged epithelial cells. Studies have shown bacteria adhere more easily to viral-infected (27,28) or nonspecifically damaged cells (27) whereas, normal cells are resistant to colonization. Bacteria which are able to colonize the membrane can then replicate and invade through the membrane to the circulation leading to bacteremia. Besides the ability to adhere to damaged cells, some bacteria, most notably Escherichia coli, can invade from the respiratory tract into the blood across an undamaged membrane under experimental conditions following aerosolization of the bacteria (2). These bacteria adhere to the plasma membrane of air capillary epithelial cells, are internalized by and transverse the cytoplasm of these cells, pass through the basement membrane and endothelial cell layer, and enter the circulation leading to bacteremia.

If the nonspecific defense mechanisms of the respiratory tract are breached, the bird responds quickly and violently to the invading agent and damage induced. Regardless of the insult, whether it be irritant, particulate, or infectious agent, the bird can respond in only a limited number of ways. In general the respiratory tract responds to injury as other body systems with degeneration and/or necrosis of the epithelium followed by acute early inflammation consisting of mucous, fibrin, and heterophil exudation and an early regenerative response composed of epithelial cell hypertrophy and hyperplasia (16). Macrophage and lymphocyte infiltration follows with hyperplastic epithelial cells differentiating and maturing into an architecturally normal lining. Often, following injury with infectious agents, lymphoid aggregates or nodules remain in the lamina propria and/or submucosa. If the inflammatory response is inadequate or the organisms are resistant to the effects of inflammation, the organisms cross the respiratory lining barrier, invade the circulation, multiply exponentially, and kill the bird by massive sepsis or damage of a critical organ (2,10,11). If it is localized in the respiratory tract, but not eliminated, abscesses, granulomas with multinucleated giant cells, and areas of fibroplasia may occur (19). If the response is adequate and the invading agent is neutralized, minimal damage occurs which must be repaired. In the trachea and bronchi, regeneration of damaged epithelial cells occurs from undifferentiated reserve cells and is rapid and usually complete in seven to 14 days (4). In parabronchi, infundibula, and air capillaries, regeneration of damaged epithelial cells occurs from granular reserve cells (type II cells) which line the atria (30). These cells must migrate long distances from the atria into air capillaries and the efficiency is questionable. It may be that extensive damage in the avian lung can not be returned to full function due to this apparent inefficient healing process. In the air sac, regeneration of the epithelium is accomplished by replication of type II air-sac epithelial cells which are normally present in low numbers in the air sac (29,36).

REFERENCES

1. Abdul-Aziz, T. A., and L. H. Arp. Pathology of the trachea in turkeys exposed by aerosol to lentogenic strains of Newcastle disease virus. Avian Dis. 27:1002-1011. 1983.

2. Ackermann M. R., and N. F. Cheville. Ultrastructural studies of the lung and its air-blood barrier in turkeys (*Meleagris gallopava*) intratracheally inoculated with *Escherichia coli*. Vet Pathol. 28:183-191. 1991.

3. Arp, L. H., and N. F. Cheville. Tracheal lesions in young turkeys infected with *Bordetella avium*. Am. J. Vet. Res. 45:2196-2200. 1984.

4. Battista, S. P., E. P. Denine, and C. J. Kensler. Restoration of tracheal mucosa and ciliary particle transport activity after mechanical denudation in the chicken. Toxicol. Appl. Pharmacol. 22:59-69. 1972.

5. Brain, J. D., and P. A. Valberg. Deposition of aerosol in the respiratory tract. Am. Rev. Respir. Dis. 120:1325-1373. 1979.

6. Coonrod, J. D., and K. Yoneda. Detection and partial characterization of antibacterial factor(s) in alveolar lining material of rats. J. Clin. Invest. 71:129-141. 1983.

7. Coonrod, J. D., R. L. Lester, and L. C. Hsu. Characterization of the extracellular bactericidal factors of rat alveolar lining material. J. Clin. Invest. 74:1269-1279. 1984.

8. Craig-Schmidt, M. C., S. A. Faircloth, P. S. Teer, J. D. Weete, and C-Y. Wu. The essential fatty acid deficient chicken as a model for cystic fibrosis. Am. J. Clin. Nutr. 44:816-824. 1986.

9. DeRosa, M. Pathogenesis of acute coliform airsacculitis in broiler chickens. Master's thesis, North Carolina State University, Raleigh, North Carolina. pp. 43-111. 1990.

10. DeRosa, M., M. D. Ficken, and H. J. Barnes. Acute airsacculitis in untreated and cyclophosphamide-pretreated broiler chickens inoculated with *Escherichia coli* or *Escherichia coli* cell-free culture filtrate. Vet. Pathol. 29:68-78. 1992.

11. Ficken, M. D., and H. J. Barnes. Acute airsacculitis in turkeys inoculated with *Pasteurella multocida*. Vet. Pathol. 26:231-237. 1989.

12. Ficken, M. D., J. F. Edwards, and J. C. Lay. Induction, collection, and partial characterization of induced respiratory macrophages of the turkey. Avian Dis. 30:766-771. 1986.

13. Ficken M. D., J. F. Edwards, and J. C. Lay. Effects of Newcastle disease virus infection on the binding, phagocytic, and bactericidal activities of respiratory macrophages of the turkey. Avian Dis. 31:888-894. 1987.

14. Ficken M. D., J. F. Edwards, J. C. Lay, and D. E. Tveter. Tracheal mucus transport rate in normal turkeys and in turkeys infected with *Bordetella avium* (*Alcaligenes faecalis*). Avian Dis. 30:154-159. 1986.

15. Ficken M. D., J. F. Edwards, J. C. Lay, and D. E. Tveter. Tracheal mucus transport rate and bacterial clearance in turkeys exposed by aerosol to La Sota strain of Newcastle disease virus. Avian Dis. 31:241-248. 1987.

16. Fletcher, O. J. Pathology of the avian respiratory system. Poult. Sci. 59:2666-2679. 1980.

17. Gordon, A. H., P. D. Arcy-Hart, and M. R. Young. Ammonia inhibits phagosome-lysosome fusion in macrophages. Nature 286:79-80. 1980.

18. Green, G. M., G. J. Jakab, R. B. Low, and G. S. Davis. Defense mechanisms of the respiratory membrane. Am. Rev. Respir. Dis. 115:479-514. 1977.

19. Gross, W. B. Pathological changes of an *Escherichia coli* infection in chickens and turkeys. Am. J. Vet. Res. 18:724-730. 1957.

20. Hayter, R. B., and E. L. Besch. Airborne-particle deposition in the respiratory tract of chickens. Poult. Sci. 53:1507-1511. 1974.

21. Jonsson, S., D. M. Musher, A. Goree, and E. C. Lawrence. Human alveolar lining material and antibacterial defenses. Am. Rev. Respir. Dis. 133:136-140. 1986.

22. McEntee, M. F., and M. D. Ficken. Blood clearance of radiolabeled gold colloid by the turkey mononuclear phagocytic system. Avian Dis. 34:393-397. 1990.

23. Mensah, G. A., and J. D. Brain. Deposition and clearance of inhaled aerosol in the respiratory tract of chickens. J. Appl. Physiol. 53:1423-1428. 1982.

24. Nagaraja, K. V., D. A. Emery, K. A. Jordan, J. A. Newman, and B. S. Pomeroy. Scanning electron microscopic studies of adverse effects of ammonia on tracheal tissues of turkeys. Am. J. Vet. Res. 44:1530-1536. 1983.

25. Nagaraja, K. V., D. A. Emery, K. A. Jordan, V. Sivanandan, J. A. Newman, and B. S. Pomeroy. Effect of ammonia on the quantitative clearance of *Escherichia coli* from lungs, air sacs, and livers of turkeys aerosol vaccinated against *Escherichia coli*. Am. J. Vet. Res. 45:392-395. 1984.

26. Prantner, M. M., B. G. Harmon, J. R. Glisson, and E. A. Mahaffey. Septicemia in vaccinated and nonvaccinated turkeys inoculated with *Pasteurella multocida* serotype A:3,4. Vet. Pathol. 27:254-260. 1990.

27. Ramphal R., P. M. Small, J. W. Shands Jr., W. Fischlschweiger, and P. A. Small Jr. Adherence of *Pseudomonas aeruginosa* to tracheal cells injured by influenza infection or by endotracheal intubation. Infect. Immun. 27:614-619. 1980.

28. Sanford B. A., A. Shelokov, and M. A. Ramsay. Bacterial adherence to virus-infected cells: a cell culture model of bacterial superinfection. J. Infect. Dis. 137:176-181. 1978.

29. Smith, J. H., J. L. Meier, C. Lamke, P. J. G. Neill, and E. D. Box. Microscopic and submicroscopic anatomy of the parabronchi, air sacs, and respiratory space of the Budgerigar (*Melopsittacus undulatus*). Am. J. Anat. 177:221-242. 1986.

30. Smith, J. H., J. L. Meier, P. J. G. Neill, and E. D. Box. Pathogenesis of *Sarcocystis falcatula* in the budgerigar. II. Pulmonary pathology. Lab. Invest. 56:72-84. 1987.

31. Stearns, R. C., G. M. Barnas, M. Walski, and J. D. Brain. Deposition and phagocytosis of inhaled particles in the gas exchange region of the duck, *Anas platyrhynchos*. Respir. Physiol. 67:23-36. 1987.

32. Swayne, D. E., M. D. Ficken, and J. S. Guy. Immunohistochemical demonstration of influenza A nucleoprotein in lungs of turkeys with natural and experimental influenza respiratory disease. Avian Pathol. 21:547-557. 1992.

33. Toth, T. E., and P. B. Siegel. Cellular defense of the avian respiratory tract: paucity of free-residing macrophages in the normal chicken. Avian Dis. 30:67-75. 1986.

34. Toth, T. E., H. Veit, W. B. Gross, and P. B. Siegel. Cellular defense of the avian respiratory system: protection against *Escherichia coli* airsacculitis by *Pasteurella multocida*-activated respiratory phagocytes. Avian Dis. 32:681-687. 1988.

35. Toth, T. E., R. H. Pyle, T. Caceci, P. B. Siegel, and D. Ochs. Cellular defense of the avian respiratory system: influx and nonopsonic phagocytosis by respiratory phagocytes by *Pasteurella multocida*. Infect. Immun. 56:1171-1179. 1988.

36. Trampel, D. W., and O. J. Fletcher. Light microscopic, scanning electron microscopic, and histomorphometric evaluation of *Mycoplasma gallisepticum*-induced airsacculitis in chickens. Am. J. Vet. Res. 42:1281-1289. 1981.

Avian Respiratory Immunity and Immunosuppression[1]

J. M. Sharma

In birds, as in mammals, lymphocytes originate in the bone marrow and are carried via the circulation to the primary lymphoid organs. The primary lymphoid organs regulate the differentiation and maturation of the two major populations of lymphocytes i.e. B cells that produce antibodies and T cells that are involved in cellular immunity. B cells undergo maturation in the bursa of Fabricius and T cells in the thymus. Mature lymphocytes leave the primary lymphoid organs and populate secondary lymphoid organs. The name secondary lymphoid organ refers to lymphoid cell aggregates that are scattered through the body. In birds, these include the spleen and lymphoid cell infiltrations in skin, gastrointestinal tract, urinogenital tract and the respiratory tract. In general, the actual immune reactions to foreign antigens takes place in the secondary lymphoid organs and not in the primary lymphoid organs. Thus, when pathogens are inhaled, the first immune cells to become exposed are the immune cells resident in the respiratory tract. These cells mount an immune reaction. If the antigen cannot be contained by the local defense mechanisms, immune cells located in other secondary lymphoid organs are likely to get stimulated resulting in a systemic immune response.

LOCAL IMMUNE RESPONSES

A number of studies have shown that exposure of the respiratory tract to pathogens results in local production of antibodies. These antibodies can be recovered from saliva, tears, nasal secretions or lavages of the trachea and lung (1,3,7,11,13,14). Generally, the classes of antibodies present in the serum can also be recovered from tracheal washings. Thus, IgM and IgG antibodies are commonly recovered from the respiratory tract. Because serum and local antibodies often occur concurrently, there is some question that the antibodies recovered from tracheal washings may be transduced from the serum. Chicks hatching from dams immunized with infectious bronchitis virus (IBV) had maternally derived serum antibodies that also transduced to the trachea (16). Despite possible leakage of some antibody from the circulation into the respiratory tract, there is evidence that local antibody production occurs in the respiratory tract. Respiratory secretions often contain IgA antibody that is a local secretory antibody not commonly found in the serum. Furthermore, *in vitro* tracheal organ cultures have been shown to secrete antibody in the culture medium (12). Chickens exposed to IBV have an increased number of Ig-bearing cells in the lamina propria of the trachea indicating local antibody production (19). The Harderian gland, a secondary lymphoid organ of the respiratory tract, is a rich source of local specific antibody (5,17).

Antibody present in the respiratory tract likely plays a role in disease resistance. The antibody may neutralize the infectious agent and thus may inhibit the replication and the spread of the agent. The beneficial effects of local antibody were especially apparent in IBV and mycoplasma infections (3,4) but not in infection with infectious laryngotracheitis virus (8).

Local cellular immune responses in the respiratory tract have not been examined in detail. Although T cells and other immune cells including natural killer (NK) cells and macrophages have been detected at the site of infection in the respiratory tract, the role of these cells in local

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Local cellular immune responses in the respiratory tract have not been examined in detail. Although T cells and other immune cells including natural killer (NK) cells and macrophages have been detected at the site of infection in the respiratory tract, the role of these cells in local defense is not clear. Infection with NDV resulted in an increase in the number of T cells in the Harderian gland of chickens and these cells responded normally to T cell mitogens (15). T cells recovered from the respiratory tract of chickens infected with IBV did not have detectable specific cytotoxic activity (22). Infection of the respiratory tract with viruses may induce local production of interferon (10,21). The immunomodulatory effects of interferon and possible involvement of other cytokines in respiratory tract infection are not known.

There is some evidence that macrophages activated by local infections may exhibit enhanced phagocytic activity (24). Under normal circumstances, avian respiratory tract contains very few resident macrophages (23). However, when chickens were given intratracheal injections of *Pasteurella multocida*, there was a rapid influx of macrophages into the respiratory tract (24). Further, the newly arrived macrophages were about three times more phagocytic than the macrophages recovered from the respiratory tract of normal hatchmates. The activated macrophages had protective ability because chickens injected with *P. multocida* were better protected against an air sac challenge with virulent *Escherichia coli* than the chickens that did not receive *P. multocida*.

EFFECT OF IMMUNOSUPPRESSION ON RESPIRATORY TRACT IMMUNITY

A number of stresses and infectious agents may compromise immune competence in poultry. Generalized immunosuppression may also affect respiratory tract immunity (2,6,18,20). Bothantibody response and cellular immunity may be affected. Infection of chickens with infectious bursal disease virus (IBDV), a naturally occurring immunosuppressive virus of chickens, resulted in reduced local immune response to IBV in the respiratory tract (20). Recently, it was shown that infection of IBDV-exposed birds with IBV resulted in normal levels of IgM but reduced levels of IgG and IgA (22). In addition, birds given IBDV and IBV had elevated levels of respiratory NK cells than the birds that received IBV alone. In another study (6), chickens were exposed to IBDV and then injected with inactivated *Brucella abortus* and sheep erythrocytes. The primary antibody titers against both antigens were lower in the extracts of the Harderian gland of IBDV-exposed chickens than in those of IBDV-free chickens.

Infection with Newcastle disease virus (NDV) may compromise macrophage function in the respiratory tract and thus increase susceptibility to bacterial infections. Turkey poults given the La Sota strain of NDV or Freund's adjuvant showed an increased influx of macrophages in the respiratory tract (9). However, macrophages induced by NDV had reduced ability to phagocytize antibody coated sheep erythrocytes and to lyse *E. coli*. Macrophages induced by the Freund's adjuvant were functionally intact.

CONCLUSIONS

The secondary lymphoid cell populations contained within the respiratory tract respond vigorously to antigenic stimulation. Both antibody and cellular responses are initiated and these responses may be important in protection against respiratory pathogens . The Harderian gland contributes significantly to the local antibody supply. IgM, IgG and IgA antibodies have been recovered from respiratory tract washings. T cells, NK cells and macrophages have been identified in the respiratory tract although the role of these cells in local cellular immune defense is not clear. Virus exposure has been shown to generate interferon in the respiratory

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REFERENCES

1. Aikken, I. D., S. H. Parry, J. R. Powell, and B. D. Survashe. Local immunity in Newcastle disease some recent experiments. Developments in Biological Standardization. 33:302-308. 1976 (S. Kager, Basel).

2. Anderson, D. P., C. W. Beard, and R. P. Hanson. Influence of poultry house dust, ammonia and carbon dioxide on the resistance of chickens to Newcastle disease virus. Avian Dis. 10:177-188. 1966.

3. Avakian, A. P., and D. H. Ley. Protective immune response to *Mycoplasma gallisepticum* demonstrated in respiratory tract washings from *M. gallisepticum*-infected chickens. Avian Dis. 37:697-705. 1993.

4. Cook, J. K. A., K. Otsuki, N. R. D. S. Martins, M.M. Ellis, and M. B. Huggins. The secretory antibody response of inbred lines of chickens to avian infectious bronchitis virus infection. Avian Path. 21:681-692. 1992.

5. Davelaar, F. G., A. Noordzij, and J. A. van der Donk. A study on the synthesis and secretion of immunoglobulins by the Harderian gland of the fowl after eyedrop vaccination against infectious bronchitis at 1-day-old. Avian Path. 11:63-79. 1982.

6. Dohms, J. E., and J. S. Jaeger. The effect of infectious bursal disease virus infection on local and systemic antibody responses following infection of 3-week-old broiler chickens. Avian Dis. 32:632-640. 1988.

7. Ewert, D. L., B. O. Beyer, and C. S. Eidson. Local antibody response in chickens: analysis of antibody synthesis to Newcastle disease virus by solid-phase radioimmunoassay and immunofluorescence with class-specific antibody for chicken immunoglobulin. Infect. Immun. 24:269-275. 1979.

8. Fahey, K. J., and J. J. York. The role of mucosal antibody in immunity to infectious laryngotracheitis virus in chickens. J. Gen. Virol. 71:2401-2405. 1990.

9. Ficken, M. D., J. F. Edwards, and J. C. Lay. Effects of Newcastle disease virus infection on the binding, phagocytic, and bactericidal activities of respiratory macrophages of the turkey. Avian Dis. 31:888-894. 1987.

10. Gelb, J., C. S. Eidson, O. J. Fletcher, and S. H. Kleven. Studies on interferon induction by infectious bursal disease virus (IBDV). II. Interferon production in White Leghorn chickens infected with an attenuated or pathogenic isolate of IBDV. Avian Dis. 23:634-645. 1979.

11. Gillette, K. G. Local antibody response to avian infectious bronchitis: virus-neutralizing antibody in tracheobronchial secretions. Avian Dis. 25:431-443. 1981.

12. Gomez, L., and L. G. Raggi. Local immunity to avian infectious bronchitis in tracheal organ culture. Avian Dis. 18:346-368. 1974.

13. Hawkes, R. A., J. H. Darbyshire, R. W. Peters, A. P. A. Mockette, and D. Cavanagh. Presence of viral antigens and antibody in the trachea of chickens infected with avian infectious bronchitis virus. Avian Path. 12:331-340. 1983.

14. Holmes, H. C. Neutralizing antibody in nasal secretions of chickens following administration of avian infectious bronchitis virus. Archiv fur die gesamte Virusforschung 43:235-241. 1973.

15. Maslak, D. M., and D. L. Reynolds. Mitogenic responses of the head-associated lymphoid tissue of the chicken. Submitted, 1993.

16. Mockett, A. P. A., K. A. Cook, and M. B. Huggins. Maternally derived antibody to infectious bronchitis virus: its detection in chicken trachea and serum and its role in protection. Avian Pathol. 16:407-416. 1987.

17. Mueller, A. P., K. Sato, and B. Glick. The chicken lacrimal gland, gland of Harder, caecal tonsil, and accessory spleens as sources of antibody producing cells. Cellular Immunol. 2:140-152. 1971.

18. Nagaraja, K. V., D. A. Emery, K. A. Jordan, V. Sivanandan, J. A. Newman, and B. S. Pomeroy. Effect of ammonia on the quantitative clearance of *Escherichia coli* from lungs, air sacs, and livers of turkeys aerosol vaccinated against *Escherichia coli*. Am. J. Vet. Res. 45:392-395. 1984.

19. Nakamura, K., J. K. A. Cook, K. Otsuki, M. B. Huggins, and J. A. Frazier. Comparative study of respiratory lesions in two chicken lines of different susceptibility infected with infectious bronchitis virus: histology, ultrastructure and immunohistochemistry. Avian Pathol. 20:241-257. 1991.

20. Naqi, S. A. Effect of infectious bronchitis virus on local immune responses in the respiratory and alimentary tracts of chickens. Abstract. 126th AVMA mtg., Orlando, Florida. 1989.

21. Sharma, J. M. *In situ* production of interferon in tissues of chickens exposed as embryos to turkey herpesvirus and Marek's disease virus. Am. J. Vet. Res. 50:882-886. 1989.

22. Thompson, G., S. Naqi, and B. Bauman. Respiratory immunity to IBV in immunocompetent and immunocompromised chickens. Proc. Xth World Vet. Poultry Assoc. Congress. Sydney, Australia, August 16-19. 1993. Abstract No. 115, page 178.

23. Toth, T. E., and P. B. Siegel. Cellular defense of the avian respiratory tract: parity of free-residing macrophages in the normal chicken. Avian Dis. 30:67-75. 1986.

24. Toth, T. E., H. Veit, W. B. Gross, and P. B. Siegel. Cellular defense of the avian respiratory system: Protection against *Escherichia coli* airsacculitis by *Pasteurella multocida*-activated respiratory phagocytes. Avian Dis. 32:681-687. 1988.

Biology and Epidemiology of Infectious Bronchitis

David Cavanagh

Infectious bronchitis virus (IBV) is one of a dozen or so known species in the genus Coronavirus, other members including porcine transmissible gastroenteritis virus and feline infectious peritonitis virus. Each virus particle has two glycoproteins in the lipid envelope. One of these proteins (S) forms the large surface projections or spikes which give negatively-stained particles their "corona-(crown)like" appearance. The other glycoprotein (M) is much smaller and only 20 or so of its amino acids are exposed on the virus surface. Within the lumen of the particle is the viral genome. This comprises a single piece of single-stranded RNA (27,600 bases) and is surrounded by the nucleocapsid (N) protein. There is a fourth protein, sM, which is associated with the membrane but otherwise little is known about it.

Coronaviruses can be placed into three groups, based on the degree of sequence identity of their proteins, with corresponding antigenic relationships within groups. Infectious bronchitis virus is the sole member of one of these groups. Not to be outdone, however, IBV exhibits extensive sequence variation, especially within the S protein. This protein plays a crucial role in two processes which are very relevant to the poultry industry. Firstly, this protein is the major inducer of protective immunity. Secondly, it induces the virus-neutralizing (VN) antibodies which not only may play an important role in immunity but are the basis of the virus neutralization tests which have been used for many years to differentiate variants.

The S protein is produced as a protein of approximately 1,160 amino acids which is cleaved into two parts, S1 and S2, comprising about 540 and 620 amino acids, respectively. It is the S1 part of the molecule which induces VN antibodies. It is also S1 which is the most variable part of the protein. Whereas the S2 part of S, as with the other virus proteins, varies among isolates by up to about 10%, the S1 of some isolates differ by 48%, differences of 20% or so being common. This variation in S1 has two important practical consequences. Firstly, the failure of a given vaccine strain to protect against all IBV field viruses and, secondly, the problem of virus strain identification. In order to be able to advise on the best prophylaxis measures, it is necessary to characterise new isolates. For many years this has been done by VN tests, resulting in the identification of many serotypes of IBV.

There are several drawbacks to the VN approach of IBV characterization, one being that the results of VN tests can be equivocal, because of lack of reciprocity. In addition we have found that some isolates which are very closely related in sequence (>95% identity in S1) and which give good cross-protection, nonetheless behave in VN tests as different serotypes. Many "classical" North American IBV strains have been sequenced within the last two years and it would be interesting to compare their degree of sequence variation with the extent of cross-protection.

What might be done to improve the accuracy of IBV strain identification? Also, will it be possible to improve the predictions that we make, with respect to vaccine choice and vaccination protocol, by using alternative diagnostic techniques? Two non-serum based approaches have been investigated in recent years, monoclonal antibodies (MAbs) and DNA technology.

The first detailed analysis of IBV strains using MAbs was done by Guus Koch and colleagues in the Netherlands who raised MAbs principally against Dutch strains isolated in the late 1970's. They used a double-antibody sandwich ELISA (DAS-ELISA) to test the MAbs with a large number of Dutch and UK isolates from the late 1970's, early 1980's and with several American strains. This showed that many of the European isolates were closely

related, each member of the group binding most of the 16 VN MAbs used. Some other Dutch strains, isolated at the same time, bound very few of the MAbs. Sequencing has confirmed that the MAb-related isolates had very similar S1 sequences, whereas the MAb-unrelated strains had extensive differences in S1. Monoclonal antibodies against several strains of IBV have been developed in the USA for diagnostic and epidemiological purposes and used in several laboratories (Collison *et al.*, Texas A & M University; Gelb *et al.*, University of Delaware; Naqi *et al.*, Cornell University). Attempts have been made to identify a small number of "key" MAbs which can differentiate one serotype from another; this promises to be a useful approach. However, with IBV there are always exceptions. Thus Naqi and colleagues examined an isolate which bound two VN MAbs, previously thought to be specific to the Arkansas 99 (Ark 99) and Massachusetts (Mass) serotypes, respectively. The isolate (CU-T2) bound both Mabs.

An alternative approach to virus characterization is to examine its nucleic acid. The approach adopted for IBV, and many other viruses, in recent years has been to use the reverse transcriptase/polymerase chain reaction (RT/PCR) as a starting point. Briefly, the enzyme RT is used to make a DNA copy of a selected part of the IBV genome, which is RNA. Subsequently the PCR is used to amplify the DNA to give usable amounts. It is not necessary to purify the RNA prior to this procedure. One hundred microliters of allantoic fluid from an infected embryo, or the RNA recovered from a tracheal swab, is sufficient for several PCR reactions. In order to amplify the genes of choice, one makes synthetic oligonucleotides which correspond to IBV sequence. Typically these oligonucleotides would be 20-25 nucleotides in length. In order to guarantee detection of any IBV strain, one requires oligonucleotides which correspond to highly conserved sequences, present in every strain. One cannot guarantee this but some oligonucleotide pairs have been made which would appear to be "universal" for IBV.

Once the presence of IBV has been confirmed by the RT/PCR, the DNA can be sequenced, perhaps the ultimate in virus characterization. As always with IBV, there are a couple of snags. One is that the gene of most interest, S1, is so variable that it can be difficult to produce universal oligonucleotides to prime sequence reactions. This problem is not insurmountable but it makes analysis of IBV more difficult that some other avian viruses. The other snag arises because IBV undergoes recombination; if there is a mixed infection, some of the progeny may have sequence from both parents. There is now lots of circumstantial evidence, from sequence data of field isolates, including the CU-T2 strain mentioned above (Naqi *et al.*, Cornell University) and others (Collison *et al.*, Texas A & M University) that many IBV strains are recombinants. Since the N gene is more conserved than S1, it would be convenient to examine only N sequence. However, two isolates could have very similar N genes but very different S1 genes, and vice versa. Therefore, for a thorough characterization of strains it would be best to sequence part of two genes, e.g. S1 and N, the end of N being about 2,000 nucleotides from the beginning of N.If recombination had occurred at some time within this intervening region then this might be detected by sequencing parts of both genes. If two isolates had the same or very similar sequence in both S1 and N then one would be justified in concluding that they were very closely related. This is important information for epidemiological studies.

It may not always be necessary to sequence the PCR products. An alternative approach that has been examined is to cleave the DNA with restriction endonucleases and then to compare the sizes of the resultant products from one strain with another. Jackwood and colleagues (University of Georgia) have done this with many strains of IBV. Their work indicates that strains of a given serotype give characteristic cleavage products of the S1 gene with selected enzymes. The products can easily and quickly be visualised after electrophoresis in gels. It remains to be seen how widely this approach can be applied.

The chances are that we shall often be one step behind IBV in the field - but that is nothing to be ashamed of with a virus as variable as IBV. The MAb and nucleic acid approaches enable us to characterise strains more accurately than in the past, while keeping in mind some of the snags described above. With regard to a question that I asked earlier, I would like to think that the information provided by these approaches will result in better decisions, or at least faster decisions, being made with regard to the choice of prophylactic measures.

Newcastle Disease: Still a Worldwide Threat to Poultry

Daniel J. King

Newcastle disease (ND) in poultry in the United States is primarily due to infections with ND virus (NDV) strains that appear to be of low virulence. Infections of poultry with similar strains are problems in other countries as well (6). These strains alone may cause productivity losses (12), but more frequently they predispose birds to losses attributed to secondary bacterial infections. Vaccination with live and/or killed NDV vaccine is widely used to prevent productivity losses attributable to these infections, but vaccination is usually ineffective in preventing NDV infection (11). A recent exception to those infections of low virulence was the occurrence of velogenic neurotropic Newcastle disease (VNND) in a range reared turkey flock in North Dakota in 1992 and was the basis for depopulation of that flock (8). This was the first evidence of virulent ND in commercial poultry in the United States since velogenic viscerotropic Newcastle disease (VVND) was diagnosed in Texas in 1974 (7). Migrating water birds are now recognized as a potential source of virulent strains of NDV, in addition to pigeons, pet birds, and infected poultry. To appreciate the potential impact of ND it is necessary to look at both the current situation in the U. S., as well as the recent history of ND world-wide.

ND is an important disease of poultry and other avian species throughout the world. Clinical manifestations vary with the age, species, and immune status of the host; the virulence of the infecting NDV strain; and the pathologic form of the disease. Clinical signs and lesions of the different disease forms remain unchanged from early descriptions and are primarily a consequence of involvement of the respiratory tract, the intestinal tract, and/or the nervous system. In addition to the domestic avian species, NDV is known to infect at least 236 species of birds (2). The nature and magnitude of the problem of ND in poultry varies among countries. Virulent forms of ND are reportable to the Office of International des Epizooties (OIE) and a list of countries with outbreaks of reportable disease are published monthly in the "OIE Bulletin" and quarterly by USDA, APHIS, VS, Emergency Programs in the "Foreign Animal Disease Report." That list invariably includes several countries with outbreaks of virulent ND. National control programs for ND vary among countries; they range from those where no vaccination is allowed and all introductions are eradicated to those that use both lentogenic and mesogenic live NDV vaccines to attempt to prevent infections with indigenous NDV strains.

All NDV isolates, regardless of species of origin, are inhibited by NDV polyclonal antiserum prepared against a reference NDV strain and their antigenic similarities are the basis for their classification as members of the avian paramyxovirus serotype 1 (PMV-1). NDV isolates can be differentiated with NDV polyclonal antiserum from the other eight avian paramyxovirus serotypes (PMV-2 through PMV-9), although there is some cross-reactivitybetween PMV-1 and PMV-3, a serotype most frequently identified in turkeys (1). Antigenic differences among NDV strains are detectable with monoclonal antibodies (MAb) and tests with a large number of NDV isolates against a battery of ten MAb have identified eleven antigenic groups. Determination of these antigenic differences has provided useful epidemiological information and a comparison of isolates within a group has in some cases identified the predominant host and the relative virulence of members of that group (1). A smaller battery of four MAb has been used similarly to characterize recent NDV isolates in the U. S. (5,8). Definitive virulence assessment or pathotyping of new isolates still requires embryonated egg and chicken inoculations as described below.

Characterization of the virulence of NDV strains requires laboratory evaluation because the immune status and susceptibility of different hosts may modify the clinical picture from that seen in inoculated susceptible chickens. The characterization is routinely done by procedures that include: inoculation of embryonated chicken eggs to determine a mean embryo death time (MDT), inoculation of one-day-old chicks to determine an intracerebral pathogenicity index (ICPI), inoculation of six-week-old chickens intravenously to determine an intravenous pathogenicity index (IVPI), and/or intracloacal inoculation of 6- to 8-week old chickens to determine virulence and to distinguish tropism of viscerotropic velogens by demonstration of characteristic lesions. The more virulent viruses typically have shorter MDTs and higher lethality which results in higher ICPIs and IVPIs (1). Results of these tests are used to classify isolates as lentogens, mesogens, and velogens, i.e. low, moderate, and high virulence, respectively. The ICPI is useful to distinguish mesogens and velogens from lentogens and the IVPI to differentiate mesogens from velogens. Of these tests, the MDT is used universally. In the U. S. the intracloacal inoculation has been used more frequently than the ICPI and IVPI because the intracloacal inoculation results in more consistent intestinal lesion development for differentiation of viscerotropic and neurotropic velogenic viruses, an important distinction during the outbreak in Calif. in 1971 through 1973 (11) and in characterizing similar exotic NDV isolates from pet birds. The current threat of virulent NDV comes from various sources.

Migrating water birds. In 1992, there was an extensive die-off of cormorants and other water birds in the Upper Midwest region (North Dakota east to New York and south to Nebraska) of the U.S. This was the first die-off due to NDV in wildlife in the U.S. One turkey flock on range near a cormorant die-off became infected, showed signs of neurologic disease and mortality, and was depopulated. The NDV isolates from cormorants and from turkeys were characterized as VNNDV (8). A similar outbreak occurred in water birds in Canada in 1990, but there was no evidence of infection of poultry from that outbreak (13). The migration patterns of cormorants covers the Eastern U.S. from Canada to the Gulf of Mexico. The virulence of these isolates is in contrast to the lentogenic NDV isolates collected from migratory waterfowl in the Atlantic flyway (10).

Pet birds, particularly psittacines. Since the VVND outbreak in California in 1971, VVNDV has been isolated from birds in quarantine or from confiscated smuggled birds in every year except 1978 and 1990 (7,8).

Poultry. VNNDV has been identified in several backyard and commercial flocks in 1993 in the Netherlands, Spain, Switzerland, Germany, Luxembourg, and Belgium. Velogenic NDV was also reported from countries in Asia and Africa in 1993 (OIE Reports) .

Pigeons. In the mid-1980s NDV was isolated from pigeons (feral pigeon and pigeon lofts) in the U. S. (9) and in several countries in the European Union. No poultry were infected in the U. S., but in England chicken flocks were infected, presumably from feed contaminated by large numbers of NDV infected pigeons. Pigeons were considered to be source of those NDV infections in England because monoclonal antibody binding patterns were similar for the chicken and pigeon isolates (3). The pigeon isolates had long MDT typical of lentogenic NDV, were inconsistent in producing disease in chickens except when inoculated intracerebrally in day-old birds (9), but produced disease and death in pigeons and with adaptation were virulent for chickens (3). The biological properties of the pigeon isolates differ from known virulent strains and are considered of minimum risk to poultry. Consequently, in the U. S., there is no regulatory program for control of this virus other than recommending vaccination with the vaccine licensed by the USDA for use in pigeons.

The virulence classification continues to evolve. Recently the European Union has adopted a standard that NDV strains with an ICPI > 0.7 will be classified as virulent (4). Both mesogenic viruses, such as Roakin strain which is used as a vaccine in some countries, and velogenic viruses would be classified as virulent by that standard.

The transmission of NDV from water birds to turkeys in 1992 is simply the most recent of past events that resulted in virulent NDV infections of poultry. That event serves as a reminder that continued vigilance is needed to prevent the losses that are the consequence of such occurrences. It is extremely important that biosecurity measures be continuously employed to prevent direct or indirect contacts between other bird species and commercial poultry.

REFERENCES

1. Alexander, D. J. Newcastle disease. In: A laboratory manual for the isolation and identification of avian pathogens, 3rd ed. H. G. Purchase, L. H. Arp, C. H. Domermuth, and J. E. Pearson, eds. American Association of Avian Pathologists, Kennett Square, Pennsylvania. pp. 114-120. 1989.

2. Alexander, D. J. Newcastle disease and other paramyxovirus infections. In: Diseases of poultry, 9th ed. B. W. Calnek, H. J. Barnes, C. W. Beard, W. M. Reid, and H. W. Yoder, Jr. Iowa State University Press, Ames, Iowa. pp. 496-519. 1991.

3. Alexander, D. J., G. W. C. Wilson, P. H. Russell, S. A. Lister, and G. Parsons. Newcastle disease outbreaks in fowl in Great Britain during 1984. Vet. Rec. 117: 429-434. 1985.

4. Council Directive 92/66/EEC of 14 July 1992 introducing Community measures for the control of Newcastle disease. Official Journal of the European Communities. No. L 260. pp. 1-20. 5 September 92.

5. Lana, D. P., D. B. Snyder, D. J. King, and W. W. Marquardt. Characterization of a battery of monoclonal antibodies for differentiation of Newcastle disease virus and pigeon paramyxovirus-1 strains. Avian Dis. 32:273-281. 1988.

6. Meulemans, G., M. Gonze, D. Morales, M. Decaesstecker, and L. Lagrange. Isolation, identification and characterization of PMV-1 strains from broiler chickens. In: Workshop on Avian Paramyxoviruses, E. F. Kaleta and U. Heffels-Redmann, Proceedings eds. Institut für Geflügelkrankheiten, Justus-Liebig-Universität, Giessen, Germany. pp. 128-138. 1992.

7. Mixson, M. Velogenic viscerotropic Newcastle disease. In: Report of the Committee on Transmissible Diseases of Poultry and other Avian Species, R. H. McCapes, Chairman. Proceedings 94th Annual Meeting of the United States Animal Health Association, Denver, Colorado. p. 324. 1990.

8. Mixson, M. A. and J. E. Pearson. Newcastle Disease. In: Report of the Committee on Transmissible Diseases of Poultry and other Avian Species, R. H. McCapes, Chairman. Proceedings 96th Annual Meeting of the United States Animal Health Association, Louisville, KY. pp. 357-363. 1992.

9. Pearson, J. E., D. A. Senne, D. J. Alexander, W. D. Taylor, L. A. Peterson, and P. H. Russell. Characterization of Newcastle disease virus (avian paramyxovirus-1) isolated from pigeons. Avian Dis. 31: 105-111. 1987.

10. Rosenberger, J. K., S. Klopp, and W. C. Krauss. Characterization of Newcastle disease viruses isolated from migratory waterfowl in the Atlantic flyway. Avian Dis. 19:142-149. 1975.

11. Utterback, W. W., and J. H. Schwartz. Epizootiology of velogenic viscerotropic Newcastle disease in Southern California, 1971-1973. J. Am. Vet. Med. Assoc. 163:1080-1088. 1973.

12. Westbury, H. A., G. Parsons, and W. H. Allan. Comparison of the residual virulence of Newcastle disease vaccine strains V4, Hitchner B1 and La Sota. Austral. Vet. J. 61: 47-49. 1984.

13. Wobeser, G., F. A. Leighton, R. Norman, D. J. Myers, D. Onderka, M. J. Pybus, J. L. Neufeld, G. A. Fox, and D. J. Alexander. Newcastle disease in wild water birds in western Canada, 1990. Canadian Veterinary Journal - Revue Veterinaire Canadienne. 34(6): 353-359. 1993.

Virulence Assessment of the Avian Influenza Virus

Max Brugh, Jr.

Current Understanding of the Laryngotracheitis Problem

James S. Guy

Laryngotracheitis (LT) is an acute herpesviral respiratory tract infection of chickens that may result in severe production losses due to mortality and decreased egg production. During recent years the incidence and economic importance of LT has increased dramatically in the United States. The reason for recent resurgence of the disease has not been conclusively determined. However, observations and experimental studies during recent outbreaks in North Carolina suggest causal involvement of modified-live (ML) vaccine viruses in these outbreaks.

HISTORY

LT was first described in 1925 in a poultry flock in Rhode Island, but probably existed in North America for many years prior to this. In 1934 it was recognized that chickens could be protected against LT by vaccination via the cloacal route using virulent field strains of LT virus. Subsequently, it was demonstrated that immunity could be provided by vaccination of chickens via infraorbital sinuses, intranasal instillation, feather follicles and drinking water using naturally occurring, low-virulence LT strains, or viruses modified in their ability to cause disease by prolonged passage in embryonated chicken eggs (chicken-embryo-origin) or cell cultures (tissue-culture-origin). The incidence of LT decreased dramatically during the 1960's and 1970's, probably as a result of improvements in biosecurity, and development of improved vaccines. Changing management practices (vertical integration and "all-in, all-out" production methods) have greatly improved biosecurity and probably contributed greatly to the decreased incidence of the disease.

CHARACTERISTICS OF THE VIRUS

Laryngotracheitis virus, also known as Gallid herpesvirus 1, is an alphaherpesvirus that possesses a double-stranded DNA genome with a molecular weight of approximately 100 x 106 (8). The virus also is composed of approximately 24 proteins, including 7 glycoproteins (11). The glycoproteins are located on the surface of the virion, within the viral envelope.

Like other herpesviruses, LT virus tends to persist in naturally-infected and vaccinated chickens (2), resulting in "carriers" which subsequently may transmit the virus to other birds. Field outbreaks of LT have occurred following contact between unvaccinated chickens and chickens vaccinated several weeks previously, indicating that vaccinated birds may shed virus for long periods.

LT viruses vary in their ability to cause disease. Highly virulent strains cause severe disease and mortality in chickens, whereas strains of low virulence cause mild clinical signs or subclinical infection.

LT occurs primarily in chickens; however, the disease has been described in pheasants, pheasant-chicken crosses, and pea fowl; young turkeys have been experimentally infected. Starlings, sparrows, crows, doves, ducks, pigeons, and guinea fowl are refractory to infection.

The usual route of infection is through the upper respiratory tract. The virus replicates primarily in the trachea and larynx, and clinical disease, if it occurs, is the result of massive destruction of cells in these tissues. Characteristic clinical signs include nasal discharge, moist

rales, coughing and gasping; however, this is dependent upon the strain of LT virus and the immunologic status of the bird. Other portals of entry include contact of virus with the eyes and ingestion of the virus. Mechanical transmission of the virus may occur via contaminated equipment, clothing, boots, and litter.

CONTROL

Currently, management practices emphasizing biosecurity, and vaccination utilizing modified-live (ML) vaccine viruses, are the methods used to prevent and control LT. Since both natural infection and vaccination have been shown to produce "carrier" birds, it is extremely important that susceptible chicken flocks are not exposed to vaccinated or previously infected chickens. Mixing of stock should be done only when a complete history of the birds is available, and it is absolutely certain that potential LT "carriers" are not present. Sanitation procedures which include disinfection of equipment, boots, and clothing, and proper disposal of litter and carcasses are essential components of LT control.

Vaccination of susceptible chickens is widely practiced in areas experiencing LT outbreaks. Immunization of chickens may be accomplished by administration of ML LT vaccine viruses by eye drop, intranasally, and orally through drinking water. While the drinking water route is the simplest method of vaccine administration, this route has been shown to result in a high proportion of chickens that fail to develop protective immunity (9). Vaccine spraying is not recommended because this route may cause severe reactions (3).

Several precautions are necessary to provide safe and effective immunization. These include: 1) ensuring that each bird receives an adequate concentration of virus, and 2) institution of strict biosecurity measures to ensure that vaccine virus is not spread to neighboring non-vaccinated flocks. Vaccine concentration is determined by the manufacturer for specific routes of administration; therefore, alternate routes may not provide adequate dosage. This is the principal reason that oral vaccination is not approved by the USDA. Care also must be exercised in handling ML LT vaccine products; to retain virus infectivity they should be kept in a cool dark place at all times prior to use.

In the future, control of LT may be facilitated by the use of recombinant virus vaccines. A recombinant, virus-vectored vaccine composed of a herpesvirus of turkeys (HVT) which expresses a LT glycoprotein recently was shown to induce protective immunity (10). Such vaccines could conceivably produce immunity without the adverse reactions sometimes associated with current ML LT vaccine viruses (e.g. vaccine reactions, development of the carrier state). Alternatively, LT could be controlled by eradication; plans for LT eradication recently were developed and approved by the United States Animal Health Association (12).

LARYNGOTRACHEITIS OUTBREAKS IN NORTH CAROLINA

Severe outbreaks of LT occurred in central North Carolina, beginning in December 1985. The initial case occurred in a flock of unvaccinated layers subsequent to the addition of LT-vaccinated-chickens. Intensive vaccination of flocks began soon after diagnosis of this initial outbreak. Sixty-three cases of LT were diagnosed in 1986 and 77 cases in 1987.

Field isolates of LT virus were collected from field outbreaks during 1986 and 1987 and examined by DNA-restriction endonuclease (RE) analyses (DNA "fingerprinting") (5). Eighteen North Carolina field isolates were examined by the RE procedure and compared to 6 ML LT vaccine viruses, and three reference strains of LT virus. The ML LT vaccine viruses examined included 5 chicken-embryo-origin (CEO) vaccine viruses and 1 tissue-culture-origin

(TCO) vaccine virus. RE cleavage patterns of the 18 field isolates were indistinguishable from vaccine viruses; however, field isolates were readily distinguished from reference strains. These analyses suggested involvement of vaccine viruses in the LT outbreaks in North Carolina, possibly the result of reversion of vaccine viruses to parental-type virulence. However, using RE analyses, definitive evidence regarding involvement of a virus in an episode of disease is obtained only when cleavage patterns are different, not when they are the same. The possibility that a virulent field strain of LT could have the same RE pattern as an ML vaccine virus could not be ruled out. Although definitive evidence incriminating ML vaccine viruses in LT outbreaks in North Carolina was not provided by RE analyses, the failure to identify RE cleavage patterns different from vaccine viruses among the 18 isolates examined strongly implicated vaccine viruses as original sources for these viruses.

Similar studies have been conducted by other investigators (1,6,7). Field isolates collected from LT outbreaks in Georgia (1), Pennsylvania (7), and the Delmarva peninsula (6) were examined by RE analyses to determine the role of vaccine viruses in these outbreaks. In these studies, most field isolates examined, but not all, were shown to have RE cleavage patterns indistinguishable from ML vaccine viruses. While these studies identified viruses that differed from vaccine viruses, field isolates with RE patterns indistinguishable from vaccine viruses predominated. The investigators concluded that additional studies were required to determine the role of vaccine viruses in LT outbreaks.

Subsequent studies were undertaken in our laboratory to determine whether ML vaccine viruses could increase in virulence after consecutive bird-to-bird passage (4). After sequential bird-to-bird passage of a CEO vaccine virus and a TCO vaccine virus in chickens, increased virulence was observed for the CEO virus but not the TCO virus. After 10 chicken passages, the CEO virus possessed virulence comparable with a highly virulent reference strain of LT virus. These findings demonstrated that the CEO virus examined in this study, and possibly other CEO viruses, are not stable with respect to virulence properties and thus may be causally involved in disease outbreaks. The failure to detect increased virulence in the TCO virus may have reflected the conditions of the study, particularly a limited number of passages in birds and/or a greater degree of attenuation of this vaccine virus compared with the CEO virus.

Reversion to parental-type virulence is a well recognized safety concern for all ML vaccine viruses and has been demonstrated for several different ML vaccines. It is possible that reversion to virulence of ML LT vaccine viruses may occur due to vaccine virus spread from vaccinated to unvaccinated chickens, with the virus increasing in virulence as it undergoes sequential in vivo passages. ML LT vaccine viruses have been shown to spread from vaccinated to unvaccinated chickens; therefore, increased virulence of vaccine virus may occur in field situations due to virus spread and naturally-occurring bird-to-bird passage.

Although ample evidence is available concerning efficacy and safety of ML LT vaccines when they are initially administered to chickens, problems may arise due to improper vaccine administration (e.g. drinking water) that fails to provide immunity to all the birds in a flock, and when biosecurity measures fail to prevent spread to unvaccinated flocks. These circumstances facilitate sequential bird-to-bird passage and potential reversion to virulence. Administration of vaccine virus in water has been shown to result in a high proportion of chickens that fail to develop immunity; this was the predominant method of vaccination during recent LT outbreaks in North Carolina. It is proposed that recent LT outbreaks in North Carolina were influenced by management practices that allowed bird-to-bird transmission and flock-to-flock spread of vaccine viruses that subsequently promoted reversion of these viruses to virulent forms.

In 1989, the North Carolina Department of Agriculture instituted a policy which restricted use of ML LT vaccine in North Carolina. Vaccination of chickens on multi-age breeder farms was allowed by permit; however, vaccination of other chickens was allowed only by permission of the state veterinarian. In addition, regulations required that administration of ML LT vaccine be carried out according to label directions, thus banning drinking water application. Since this policy was instituted only sporadic cases of LT have been diagnosed in North Carolina.

CONCLUSIONS

Several lines of evidence suggest involvement of ML LT vaccine viruses as causes of recent LT outbreaks in North Carolina. These include: 1) the initial outbreak occurred subsequent to addition of vaccinated chickens to an unvaccinated flock, 2) increased incidence of disease in North Carolina closely paralleled the increased usage of ML LT vaccines, 3) RE cleavage patterns of field isolates were indistinguishable from ML vaccine viruses, 4) CEO viruses were shown to increase in virulence after bird-to-bird passage, and 5) outbreaks of LT in North Carolina were effectively curtailed by legally restricting use of ML LT vaccines.

REFERENCES

1. Andreasen, J. R., J. R. Glisson, and P. Villegas. Differentiation of vaccine strains and Georgia field isolates of infectious laryngotracheitis virus by their restriction endonuclease fragment patterns. Avian Dis. 34:646-656. 1990.

2. Bagust, T. J. Laryngotracheitis (Gallid-1) herpesvirus infection in the chicken. 4. Latency establishment by wild and vaccine strains of ILT virus. Avian Path. 15:581-595. 1986.

3. Clark, J. K., G. M. Robertson, and D. A. Purcell. Spray vaccination of chickens using infectious laryngotracheitis. Aust. Vet. J. 56:425-428. 1980.

4. Guy, J. S., H. J. Barnes, and L. Smith. Increased virulence of modified-live infectious laryngotracheitis vaccine virus after bird-to-bird passage. Avian Dis. 35:348-355. 1991.

5. Guy, J. S., H. J. Barnes, and L. L. Munger. Restriction endonuclease analysis of infectious laryngotracheitis viruses: Comparison of modified-live vaccine viruses and North Carolina field isolates. Avian Dis. 33:316. 1989.

6. Keeler, C. L., J. W. Hazel, J. E. Hastings, and J. K. Rosenberger. Restriction endonuclease analysis of Delmarva field isolates of infectious laryngotracheitis virus. Avian Dis. 37:418-426. 1993.

7. Keller, L. H., C. E. Benson, S. Davidson, and R. J. Eckroade. Differences among restriction endonuclease DNA fingerprints of Pennsylvania field isolates, vaccine strains and challenge strains of infectious laryngotracheitis virus. Avian Dis. 36:575-581. 1992.

8. Kotiw, M. C., R. Wilks, and J. T. May. Differentiation of infectious laryngotracheitis virus strains using restriction endonucleases. Avian Dis. 26:718-731. 1982.

9. Robertson, G. M., and J. R. Egerton. Replication of infectious laryngotracheitis virus in chickens following vaccination. Aust. Vet. J. 57:119-123. 1981.

10. Saif, Y. M., J. K. Rosenberger, S. S. Cloud, M. A. Wild, J. K. McMillan, and R. D. Schwartz. Efficacy and safety of a recombinant herpesvirus of turkeys containing genes from infectious laryngotracheitis virus. Proceedings of the 130th Annual Meeting of the American Veterinary Medical Association, Minneapolis, p. 154. 1993.

11. York, J. J., and K. J. Fahey. Humoral and cell-mediated immune responses to the glycoproteins of infectious laryngotracheitis herpesvirus. Arch. Virol. 115:289-297. 1990.

12. Guidelines for eradication of infectious laryngotracheitis encompassing the broiler industry, table egg industry, exhibition and backyard poultry. Proceedings of 94th Annual Meeting United States Animal Health Association, p. 337-339. 1990.

Pathogenesis of Respiratory *Escherichia coli* and *Pasteurella multocida* Infections in Poultry

H. John Barnes

Respiratory disease caused by *Escherichia coli* or *Pasteurella multocida* in poultry requires three interdependent factors: pathogenicity and virulence of the causative organism, degree of exposure, and host susceptibility. The relative contribution of each not only determines disease occurrence, but also how severe the disease will be in an individual or flock. Once *E. coli* or *P. multocida* comes in contact with a susceptible bird, it must gain entrance through a portal of entry, colonize, multiply, and spread, while simultaneously evading host defense mechanisms, to produce disease. Host survival depends on containing the infection and replacing, repairing, or sequestering damaged tissue. If damaged tissue cannot be replaced, the bird must adapt to the degree of decreased function resulting from the lost tissue. When infections caused by *E. coli* or *P. multocida* are only partially controlled, localized, chronic infections may occur in the brain, eyes, oviduct, bones, joints, or other synovial structures (2,19,46,49). Such chronically infected birds are generally not productive. For the organism to survive, it must have a means of leaving the bird and coming in contact with other susceptible hosts.

VIRULENCE FACTORS

E. coli and *P. multocida* are pathogenic bacteria capable of causing severe respiratory tract and systemic infections in birds (19,87,101), as well as other localized infections beyond the scope of this review. It is likely *E. coli* infections are the most common cause of morbidity, mortality, and economic loss in poultry resulting from infectious disease. While less common, outbreaks of fowl cholera caused by *P. multocida*, can result in high morbidity and mortality, especially in turkey flocks. Both organisms are gram-negative bacteria containing potent endotoxins in their cell walls. Endotoxin provokes an acute inflammatory response causing localized tissue damage, which provides the opportunity for bacteria to gain access to the systemic circulation (22,40,41). *E. coli* is an opportunist of variable virulence that requires impaired host defenses or overwhelming exposure to produce respiratory disease, while *P. multocida* is usually a primary pathogen of greater virulence. Older birds are more susceptible to respiratory infection with *P. multocida* while younger birds are less susceptible--the reverse being more characteristic of *E. coli* infections.

Virulence of *E. coli* isolates can be determined by inoculation of chickens, day-old chicks, or embryos (2,6,42,64,81,92,96,125,128). Virulence of *P. multocida* can also be determined by inoculating experimental animals; poultry, mice, rabbits, and pigeons are highly susceptible (101). No single characteristic can be used to distinguish virulent from non-virulent strains. Virulence of *E. coli* has been associated in varying degrees with: a) presence of large plasmids (127), b) certain serotypes (O1, O2, O78 and K1, K80) (14,30,58,64,78,104,110) or genetically-related clonal groups having wide geographic distribution (67,125), c) colicin (especially ColV) production (11,35,64,122), d) presence of siderophores (aerobactin) (10,14,26,122), e) cytotoxins (8,35), f) presence of fimbriae (pili) (5,26,29,30,36,56,82,90,113,126,131), g) motility (5,126,129), h) ability to colonize or persist in the circulation, trachea, liver, or intestinal tracts (4,6,24,28,130), i) cell adhesion (14), which may be preferential for avian cells (131), j) cell invasiveness (122), k) smooth LPS (128), and l) complement (serum) resistance (32,33,92,93,94,126,128). Production of smooth colonies that are iridescent when viewed by oblique light is typical of virulent *P. multocida* strains.

These strains are encapsulated, not agglutinated by immune serum, resist complement-mediated lysis and phagocytosis, and have improved survival in phagocytes (57,99,101,119). In vivo passage rapidly increases virulence of both encapsulated and non-encapsulated *P. multocida* strains (74). Toxins and adhesins have also been suggested as *P. multocida* virulence factors but their contributions to the pathogenesis of fowl cholera are not well understood (99). Because complement resistance is highly associated with virulence of both organisms, its identification provides a reasonably accurate in vitro method for assessing virulence (32,33,77,92,93,94,126,128).

EXPOSURE AND INFECTION

Escherichia coli is a normal inhabitant of the digestive system of poultry. It can also be readily isolated from the pharynx and respiratory tracts of otherwise healthy birds (61,62), but whether or not its presence there constitutes part of the "normal" flora of the respiratory tract remains unresolved (25). *E. coli* gains access to the bird's tissues via: in ovo infection, umbilicus of newly hatched birds, digestive tract, or respiratory tract. Oral exposure of gnotobiotic birds results in septicemia and respiratory disease experimentally (28). In normal chicks and poults with a resident flora established in the gut, oral exposure to *E. coli* is unlikely to be an important portal of entry unless integrity of the digestive tract is compromised. Aerogenous exposure of the respiratory system is believed to be the most common method by which natural respiratory *E. coli* infections occur (43,48). Aerosol, intratracheal, or intra-air sac exposure are frequently used to experimentally reproduce respiratory colibacillosis. As the environment of the flock becomes increasingly contaminated, birds are constantly exposed to high numbers of *E. coli* orally and via the respiratory system to aerosols, which may contain as many as 106 cfu/g of dust (59). Outbreaks of colisepticemia often occur soon after high levels of *E. coli* are present in poultry house air (16).

Pasteurellae colonize the oropharynx and upper respiratory tract of a variety of animals following oral exposure. Turkeys in flocks that previously experienced fowl cholera remain carriers because of oropharyngeal colonization (17). Oropharyngeal colonization and rapid tissue invasion occurring within hours distinguishes virulent and avirulent *P. multocida* strains even if they are antigenically and biochemically similar (100,102). Virulent strains gain access to the circulation by penetrating through lymphoid tissue in the oropharynx or conjunctiva. Acute septicemia also may develop after susceptible turkeys are bitten by animals that are oral carriers of *P. multocida* (44). Involvement of the respiratory system in fowl cholera is more likely via the hematogenous route than from inhalation (100).

Once *E. coli* or *P. multocida* gain access to the bird's circulation, they are rapidly removed by phagocytes located primarily in the spleen and liver (3,4,7,119). Because of its greater size, the liver removes most of the bacteria from the bloodstream (3,119). Opsonization with either passively or actively acquired antibody substantially improves rate of bacterial clearance (3,57). Although organisms are cleared by phagocytosis, if intracellular killing is impaired by either the bacterium or other cause(s), a persistent intracellular infection can result (4). It has been speculated that under such circumstances, migration of infected phagocytes could be a means of bacterial spread to other areas of the body (34).

HOST SUSCEPTIBILITY

Virtually any factor that affects host defenses alters resistance to *E. coli* infections. These include a variety of respiratory, enteric, and immunosuppressive viruses (23,39,86,88,89,91,112); other respiratory bacteria, especially mycoplasmas, and protozoa

(38,48,66,76,103,107,114,120,121); intestinal microorganisms that cause mucosal damage (13,79,84); chemical and physical agents (83,85); and environmental factors (70). Synergistic, mixed infections with viruses, mycoplasmas, and *E. coli* have long been recognized in broiler chickens as causing chronic, respiratory disease (12,43,45,47,50,111). Interaction of infectious bronchitis virus and *E. coli* has been extensively studied and used to determine virulence of bacterial isolates, virus isolates, and efficacy of immunity to infectious bronchitis (14,15,20,21,43,88,89,108,132). Similar multifactorial respiratory diseases, including fowl cholera, also occur in turkeys (18,66,107).

Resistance to *E. coli* can be increased by immunity; colonization with native flora (109,123,124); increased age (43,104); moderate stress (51,52,53,54,55,68); nutrition; and genetics. Immunity is central to controlling and containing *E. coli* and *P. multocida* infections (71,75,97,101). Passively acquired maternal antibody protects chicks and poults against *E. coli* for the first few weeks of life (65,95,105). As maternal antibody levels decline, actively acquired immunity from natural exposure will develop and provide resistance. Infection of birds with partial immunity can result in localized, chronic infections of synovia (5). Use of immunostimulants is being examined, which may offer a means of augmenting naturally acquired resistance. Respiratory tract stimulation using a low virulent *P. multocida* vaccine strain confers protection against subsequent air-sac challenge with virulent *E. coli* (118).

Nutrition can play an important role in modulating colibacillosis. Antioxidant lipid-soluble vitamins A and E enhance immunity and reduce the effects of *E. coli* following experimental inoculation (63,115,116). Depression of prostaglandin synthesis by vitamin E, which leads to increased humoral immunity and phagocytosis, is considered to be the basic mechanism by which resistance is enhanced (73). ß-carotene is not as effective in promoting resistance to *E. coli* as vitamins A or E, but provides increased efficacy when combined with vitamin E in reducing mortality and hepatomegaly (117). Ascorbic acid reduces the stress response and severity of respiratory *E. coli*, however, an optimum feeding level needs to be determined based on how the birds normally respond to stress (54). The positive benefits of feeding ascorbic acid are additive with those of furaltadone, and possibly could improve effectiveness of other antimicrobials. Hypoferremia occurs following air sac inoculation of turkeys; administration of exogenous iron increases mortality, bacteremia, and severity of lesions (9). Passive immunization of turkeys against outer membrane iron-regulating proteins of a virulent O78 *E. coli* provided protection against subsequent air sac challenge (10). In contrast, Harry (60) found increased resistance to experimental colisepticemia in chicks when their diets were supplemented with high levels of ferrous sulfate. Host nutritional factors affecting fowl cholera do not appear to have been studied.

Increased resistance to *E. coli* and *P. multocida* infections also is possible through genetic selection. When broiler chickens were selected for high antibody response to *E. coli* vaccination at 10 days, they had increased resistance to challenge and greater general disease resistance compared to those selected for a low antibody response. Selection for disease resistance was highly heritable and not correlated with body weight at processing (72). A line of chickens selected for high antibody response was more sensitive to experimental *E. coli* infection but lost less weight than a line selected for low antibody response (31). When these lines were exposed to primary respiratory disease agents prior to aerosolized *E. coli*, the high antibody line had the lowest occurrence of pericarditis and mortality. Wide differences were found in susceptibility of inbred lines of chickens to experimental mixed infectious bronchitis virus/*E. coli* infection (15). Genetic resistance to *E. coli* infection appears to have a dominant autosomal pattern of inheritance unrelated to MHC antigens (15,31). It appears to derive more from resistance to the effects of predisposing primary respiratory infections than *E. coli* infection (15,53). Genetic variation in resistance of various lines turkeys to fowl cholera has

also been found. Unfortunately the line selected for increased growth was more susceptible (106). Resistance to fowl cholera in chickens is related to the major histocompatibility complex (69).

PATHOGENESIS AND LESIONS

There are few descriptions of the pathology of naturally occurring colibacillosis affecting the respiratory tract of poultry (19,47,87); most information has come from experimental studies (1,3,4,22,27,50,68,80,88,96,120). Respiratory infections with *E. coli* are classified into three syndromes: a) acute septicemia, b) subacute fibrinopurulent serositis, or c) granulomatous pneumonitis (19). Acute septicemia and subacute serositis can coexist in the same bird, especially older ones because they are more likely to survive the initial acute septicemia (87). When the mucosal surface is breached, there is an immediate, pronounced acute inflammatory response characterized initially by edema, serofibrinous exudation, and heterophil influx (1,22). Margination of thrombocytes and granulocytes occurs in small arteries. With time and dose, severity of the reaction increases and macrophages begin to appear, accompanied later by lymphocytes, plasma cells, and fibroblasts. Epithelial cells in affected areas often undergo necrosis. In the lung, parabronchi fill with inflammatory exudate to the point they may rupture (1) and there is spread to the interstitium and pleura resulting in pleuropneumonia. Pneumonia is most severe in the ventral-caudal part of the lung and adjacent to primary and secondary bronchi (87). In the air sac, exudate adheres to the eroded epithelial surface (22,50,87) and there is subepithelial acute inflammation, which results in marked thickening. Spread to other serous membranes results in a serofibrinous polyserositis involving the pericardial sac, peritoneum, other air sacs, and/or pleurae. An avian heterophilic, granulomatous response and fibrosis eventually develops, which often contains considerable inspissated protein and occasional bacterial colonies (19,87). Areas of necrosis and exudation in the air sac become organized and there is serosal re-epithelialization (19). Ascites may develop in chickens as a result of residual pulmonary damage (87).

When turkeys are aerosolized with virulent *E. coli*, the most pronounced inflammatory response occurs at the junction between the primary and secondary bronchi and ostia of the air sacs. These likely represent impaction sites where inhaled foreign material is preferentially deposited (80). Following deposition of *E. coli* in the lung, they rapidly adhere to atrial and air capillary epithelia. Adhesion is apparently independent of fimbriae, at least in turkeys, but may be related to affinities of the organism for trilaminar substance on the surface of atrial epithelial cells and surfactant covering air capillary epithelial cells. Within 30 minutes, bacteria penetrate the cells and enter adjacent interstitial tissues and blood vessels, particularly small veins in the interstitium. Passage into vessels across air capillary walls, either directly through epithelial cells or from interstitial fenestrae between adjacent parabronchi, appears to be the most significant way in which *E. coli* gains access to the host's circulation. Epithelial cell loss because of either bacterial- or inflammation-derived cytotoxic substances further reduces resistance to bacterial spread into tissues (1).

Bacterial adherence to epithelial cells and intraepithelial bacteria does not occur when the air sac is involved. Epithelial cells become swollen, vacuolated, and separated, which allows *E. coli* access to the interstitium and vasculature. These same tissue changes occur in response to cell-free culture filtrate indicating they are mediated by one or more toxins produced by the organism; not by the organism itself. The response is unchanged when heteropenia is produced by cyclophosphamide treatment, indicating inflammatory products are unlikely to be responsible for the epithelial cell changes (22).

When colibacillosis occurs secondarily to a primary infectious organism, lesions characteristic of the primary agent will be seen in addition to those caused by *E. coli*. For example, tracheal lesions are as pronounced in chickens infected with infectious bronchitis virus (IBV) as with combined infections of IBV and virulent *E. coli* (88). If focal lymphoid proliferation in the airway mucosae results from primary respiratory agents, greatest susceptibility to *E. coli* occurs when the lymphoid response is well developed (53). Lymphoid foci can serve as preferential sites for *E. coli* invasion and early inflammation (53,120), which is considered to be an example of a "local, overspecialized cellular response (53)." Even in normal turkeys, initial inflammatory changes have been noted in bronchial-associated lymphoid tissue following experimental aerosol exposure to virulent *E. coli* (80).

The lungs of birds with fowl cholera are affected early in the course of the disease following exposure to virulent *P. multocida* (98). How the organism gets to the lungs following oropharyngeal inoculation remains controversial, but evidence suggests the hematogenous route is most likely (100). Vascular lesions characterized by fibrin thrombi and serous, serofibrinous, or fibrinous exudate predominate within hours after exposure.

Heterophils progressively increase in interstitial tissues and airways. Later, necrosis of pulmonary tissues, extensive airway accumulations of exudate containing fibrin, degenerating heterophils, extracellular bacteria, and fibrinoid necrosis and vasculitis of pulmonary vessels are seen (98,99). In surviving birds, extensive areas of pulmonary necrosis persist for extended periods of time. These are sequestered by a zone of granulomatous inflammation. The response of the air sac to *P. multocida* or a cell-free culture filtrate is similar to that which results from *E. coli* (37,40).

REFERENCES

1. Ackermann, M. R., and N. F. Cheville. Ultrastructural studies of the lung of turkeys (*Meleagris gallopavo*) inoculated intratracheally with *Escherichia coli*. Vet. Pathol. 28:183-191. 1991.

2. Arp, L. H. Consequences of active or passive immunization of turkeys against *Escherichia coli* O78. Avian Dis. 24:808-815. 1980.

3. Arp, L. H. Effect of passive immunization on phagocytosis of blood-borne *Escherichia coli* in spleen and liver of turkeys. Am. J. Vet. Res. 43:1034-1040. 1982.

4. Arp, L. H., and N. F. Cheville. Interaction of blood-borne *Escherichia coli* with phagocytes of spleen and liver in turkeys. Am. J. Vet. Res. 42:650- 657. 1981.

5. Arp, L. H., and A. E. Jensen. Piliation, hemagglutination, motility, and generation time of *Escherichia coli* that are virulent or avirulent for turkeys. Avian Dis. 24:153-161. 1980.

6. Arp, L. H., C. L. G. Graham, and N. F. Cheville. Comparison of clearance rates of virulent and avirulent *Escherichia coli* in turkeys after aerosol exposure. Avian Dis. 23:386-391. 1979.

7. Baba, T. Cell-mediated immune protection in chickens against *Pasteurella multocida*. J. Med. Microbiol. 36:225-230. 1984.

8. Beutin, L., D. Geier, H. Steinrück, S. Zimmermann, and F. Scheutz. Prevalence and some properties of verotoxin (Shiga-like toxin)-producing *Escherichia coli* in seven different species of healthy domestic animals. J. Clin. Microbiol. 31:2483-2488. 1993.

9. Bolin, C. A. Effects of exogenous iron on *Escherichia coli* septicemia of turkeys. Am. J. Vet. Res. 47:1813-1816. 1986.

10. Bolin, C. A., and A. E. Jensen. Passive immunization with antibodies against iron-regulated outer membrane proteins protects turkeys from *Escherichia coli* septicemia. Infect. Immun. 55:1239-1242. 1987.

11. Bounous, D. I., R. E. Wooley, and J. Brown. Non-association of macrophage phagocytosis and oxidant stimulation with complement resistance and colicin V production by avian *Escherichia coli*. Avian Dis. 38:135-140. 1994.

12. Bradbury, J. M. Avian mycoplasma infections: prototype of mixed infections with mycoplasmas, bacteria, and viruses. Ann. Microbiol. (Inst. Pasteur) A 135:83-89. 1984

13. Bradley, R. E., and W. M. Reid. Histomonas meleagridis and several bacteria as agents of infectious enterohepatitis in gnotobiotic turkeys. Expt. Parasitol. 9:91-101. 1966.

14. Brée, A., M. Dho, and J. P. Lafont. Comparative infectivity for axenic and specific-pathogen-free chickens of O2 *Escherichia coli* strains with or without virulence factors. Avian Dis. 33:134-139. 1989.

15. Bumstead, N., M. B. Huggins, and J. K. A. Cook. Genetic differences in susceptibility to a mixture of avian infectious bronchitis virus and *Escherichia coli*. Br. Poult. Sci. 30:39-48. 1989.

16. Carlson, H. C., and G. R. Whenham. Coliform bacteria in chicken broiler house dust and their possible relationship to coli-septicemia. Avian Dis. 12:297-302. 1968.

17. Carpenter, T. E., D. C. Hirsh, R. W. Kasten, D. A. Hird, K. P. Snipes, and R.H. McCapes. *Pasteurella multocida* recovered from live turkeys: prevalence and virulence in turkeys. Avian Dis. 33:12-17. 1989.

18. Carpenter, T. E., K. P. Snipes, D. A. Hird, and D. C. Hirsh. Serologic associations between fowl cholera and other diseases. Avian Dis. 35:718-722. 1991.

19. Cheville, N. F., and L. H. Arp. Comparative pathologic findings of *Escherichia coli* infection in birds. J. Am. Vet. Med. Assoc. 173:584-587. 1978.

20. Cook, J. K. A., H. W. Smith, and M. B. Huggins. Infectious bronchitis immunity: its study in chickens experimentally infected with mixtures of infectious bronchitis virus and *Escherichia coli*. J. Gen. Virol. 67:1427-1434. 1986.

21. Cook, J. K. A., M. B. Huggins, and M. M. Ellis. Use of an infectious bronchitis virus and *Escherichia coli* model infection to assess the ability to vaccinate successfully against infectious bronchitis virus in the presence of maternally-derived immunity. Avian Pathol. 20:619-626. 1991.

22. DeRosa, M., M. D. Ficken, and H. J. Barnes. Acute airsacculitis in untreated and cyclophosphamide-pretreated broiler chickens inoculated with *Escherichia coli* or *Escherichia coli* cell-free culture filtrate. Vet. Pathol. 29:68-78. 1992.

23. Dhillon, A. S., and R. W. Winterfield. Pathogenicity of various adenovirus serotypes in the presence of *Escherichia coli* in chickens. Avian Dis. 28:147-153. 1984.

24. Dho, M., and J. P. Lafont. *Escherichia coli* colonization of the trachea in poultry: comparison of virulent and avirulent strains in gnotoxenic chickens. Avian Dis. 26:787-797. 1982.

25. Dho, M., and C. Mouline. (Qualitative and quantitative analysis of the bacterial flora in the trachea of healthy chickens). Analyse qualitative et quantitative de la flore bactérienne dans la trachée du poulet sain. Ann. Rech. Vét. 14:189-194. 1983.

26. Dho, M., and J. P. Lafont. Adhesive properties and iron uptake ability in *Escherichia coli* lethal and nonlethal for chicks. Avian Dis. 28:1016-1025. 1984.

27. Dominick, M. A., and N. F. Cheville. Pathology of cloacal bursae of gnotobiotic turkeys orally inoculated with *Escherichia coli*. Vet. Pathol. 22:262-271. 1985.

28. Dominick, M. A., and A. E. Jensen. Colonization and persistence of *Escherichia coli* in axenic and monoaxenic turkeys. Am. J. Vet. Res. 45:2331-2335. 1984.

29. Dominick, M. A., M. J. F. Schmerr, and A. E. Jensen. Expression of type 1 pili by *Escherichia coli* strains of high and low virulence in the intestinal tract of gnotobiotic turkeys. Am. J. Vet. Res. 46:270-275. 1985.

30. Dozois, C. M., J. M. Fairbrother, J. Harel, and M. Bossé. pap- and pil-Related DNA sequences and other virulence determinants associated with *Escherichia coli* isolated from septicemic chickens and turkeys. Infect. Immun. 60:2648-2656. 1992.

31. Dunnington, E. A., P. B. Siegel, and W. B. Gross. *Escherichia coli* challenge in chickens selected for high or low antibody response and differing in haplotypes at the major histocompatibility complex. Avian Dis. 35:937-940. 1991.

32. Ellis, M. G., L. H. Arp, and S. J. Lamont. Serum resistance and virulence of *Escherichia coli* isolated from turkeys. Am. J. Vet. Res. 49:2034-2037. 1988.

33. Ellis, M. G., L. H. Arp, and S. J. Lamont. Interaction of turkey complement with *Escherichia coli* isolated from turkeys. Am. J. Vet. Res. 50:1285-1289. 1989.

34. Emery, D. A., K. V. Nagaraja, V. Sivanandan, B. W. Lee, C. L. Zhang, and J. A. Newman. Endotoxin lipopolysaccharide from *Escherichia coli* and its effects on the phagocytic function of systemic and pulmonary macrophages in turkeys. Avian Dis. 35:901-909. 1991.

35. Emery, D. A., K. V. Nagaraja, D. P. Shaw, J. A. Newman, and D. G. White. Virulence factors of *Escherichia coli* associated with colisepticemia in chickens and turkeys. Avian Dis. 36:504-511. 1992.

36. Ergani, O., O. Kaya, M. Çorlu, and E. Éstanbulluogglu. Hemagglutination, hydrophobicity, enterotoxigenicity, and drug-resistance characteristics of avian *Escherichia coli*. Avian Dis. 33:631-635. 1989.

37. Ficken, M. D., and H. J. Barnes. Acute airsacculitis in turkeys inoculated with *Pasteurella multocida*. Vet. Pathol. 26:231-237. 1989.

38. Ficken, M. D., J. F. Edwards, and J. C. Lay. Clearance of bacteria in turkeys with *Bordetella avium*-induced tracheitis. Avian Dis. 30:352-357. 1986.

39. Ficken, M. D., J. F. Edwards, and J. C. Lay. Effects of Newcastle disease virus infection on the binding, phagocytic, and bactericidal activities of respiratory macrophages of the turkey. Avian Dis. 31:888-894. 1987.

40. Ficken, M. D., H. J. Barnes, and M. A. Qureshi. Acute airsacculitis in turkeys inoculated with cell-free culture filtrate of *Pasteurella multocida*. Vet. Pathol. 28:46-54. 1991.

41. Ficken, M. D., H. J. Barnes, A. P. Avakian, and D. K. Carver. Vaccination of turkeys with cell-free filtrate of *Pasteurella multocida*: effects of ultrafiltration and endotoxin removal. Avian Dis. 36:423-431. 1992.

42. Gjessing, K. M., and H. A. Berkhoff. Experimental reproduction of airsacculitis and septicemia by aerosol exposure of 1-day-old chicks using Congo red-positive *Escherichia coli*. Avian Dis. 33:473-478. 1989.

43. Goren, E. Observations on experimental infection of chicks with *Escherichia coli*. Avian Pathol. 7:213-224. 1978.

44. Gregg, D. A., L. O. Olson, and E. L. McCune. Experimental transmission of *Pasteurella multocida* from raccoons to turkeys via bite wounds. Avian Dis. 18:559-564. 1974.

45. Gross, W. B. *Escherichia coli* as a complicating factor in chronic respiratory disease of chickens and infectious sinusitis of turkeys. Poult. Sci. 35:765-771. 1956.

46. Gross, W. B. *Escherichia coli* infection of the chicken eye. Avian Dis. 1:36-41. 1957a.

47. Gross, W. B. Pathological changes of an *Escherichia coli* infection in chickens and turkeys. Am. J. Vet. Res. 18:724-730. 1957b.

48. Gross, W. B. Symposium on chronic respiratory disease of poultry. II. The role of *Escherichia coli* in the cause of chronic respiratory disease and certain other respiratory diseases. Am. J. Vet. Res. 19:448-452. 1958.

49. Gross, W. B. A synovitis caused by a strain of *Escherichia coli*. Avian Dis. 5:218-220. 1961a.

50. Gross, W. B. The development of "air sac disease". Avian Dis. 5:431-439. 1961b.

51. Gross, W. B. Combined effects of deoxycorticosterone and furaltadone on *Escherichia coli* infection in chickens. Am. J. Vet. Res. 45:963-966. 1984a.

52. Gross, W. B. Effect of a range of social stress severity on *Escherichia coli* challenge infection. Am. J. Vet. Res. 45:2074-2076. 1984b.

53. Gross, W. B. Factors affecting the development of respiratory disease complex in chickens. Avian Dis. 34:607-610. 1990.

54. Gross, W. B. Effects of ascorbic acid on stress and disease in chickens. Avian Dis. 36:688-692. 1992.

55. Gross, W. B., and P.B. Siegel. Socialization as a factor in resistance to infection, feed efficiency, and response to antigen in chickens. Am. J. Vet. Res. 43:2010-2012. 1982.

56. Gyimah, J. E., and B. Panigrahy. Adhesin-receptor interactions mediating the attachment of pathogenic *Escherichia coli* to chicken tracheal epithelium. Avian Dis. 32:74-78. 1988.

57. Harmon, B. G., J. R. Glisson, and J. C. Nunnally. Turkey macrophage and heterophil bactericidal activity against *Pasteurella multocida*. Avian Dis. 36:986-991. 1992.

58. Harry, E. G. A study of 119 outbreaks of coli-septicaemia in broiler flocks. Vet. Rec. 76:443-449. 1964.

59. Harry, E. G. The survival of *E. coli* in the dust of poultry houses. Vet. Rec. 76:466-470. 1964.

60. Harry, E. G. Increase in resistance to acute experimental coli-septicaemia in chicks given high levels of ferrous sulphate in the diet. Res. Vet. Sci. 27:175-179. 1979.

61. Harry, E. G., and L.A. Hemsley. The association between the presence of septicaemia strains of *Escherichia coli* in the respiratory and intestinal tracts of chickens and the occurrence of coli septicaemia. Vet. Rec. 77:35-40. 1965.

62. Harry, E. G., and L. A. Hemsley. The relationship between environmental contamination with septicaemia strains of *Escherichia coli* and their incidence in chickens. Vet. Rec. 77:241-245. 1965.

63. Heinzerling, R. H., C. F. Nockels, C. L. Quarles, and R. P. Tengerdy. Protection of chicks against *E. coli* infection by dietary supplementation with vitamin E. Proc. Soc. Exp. Biol. Med. 146:279-283. 1974.

64. Heller, E. D., and N. Drabkin. Some characteristics of pathogenic *Escherichia coli* strains. Br. Vet. J. 133:572-578. 1977.

65. Heller, E. D., G. Leitner, N. Drabkin, and D. Melamed. Passive immunisation of chicks against *Escherichia coli*. Avian Pathol. 19:345-354. 1990.

66. Hinz, K. H., M. Ryll, U. Heffels-Redmann, and M. Poppel. (Multicausal infectious respiratory disease of turkey poults.) Multikausal bedingte infektiose Atemwegserkrankung junger Mastputen. Deutsche Tierarztliche Wochenschrift 99:75-78. 1992.

67. Kapur, V., D. G. White, R. A. Wilson, and T. S. Whittam. Outer membrane protein patterns mark clones of *Escherichia coli* O2 and O78 strains that cause avian septicemia. Inf. Immun. 60:1687-1691. 1992.

68. Kendler, J., and E. G. Harry. Systemic *Escherichia coli* infection as a physiological stress in chickens. Res. Vet. Sci. 8:212-218. 1967.

69. Lamont, S. J., C. Bolin, and N. Cheville. Genetic resistance to fowl cholera is linked to the major histocompatibility complex. Immunogenetics 25:284-289. 1987.

70. Leitner, G., and E. D. Heller. Colonization of *Escherichia coli* in young turkeys and chickens. Avian Dis. 36:211-220. 1992.

71. Leitner, G., E. D. Heller, and A. Friedman. Sex related differences in immune response and survival rate of broiler chickens. Vet. Immunol. Immunopathol. 21:249-269. 1989.

72. Leitner, G., Z. Uni, A. Cahaner, M. Gutman, and E. D. Heller. Replicated divergent selection of broiler chickens for high or low early antibody response to *Escherichia coli* vaccination. Poultry Sci. 71:27-37. 1992.

73. Likoff, R. O., D. R. Guptill, L. M. Lawrence, C. C. McKay, M. M. Mathias, C. F. Nockels, and R. P. Tengerdy. Vitamin E and aspirin depress prostaglandins in protection of chickens against *Escherichia coli* infection. Am. J. Clin. Nutr. 34:245-241. 1981.

74. Matsumoto, M., and J. G. Strain. Pathogenicity of *Pasteurella multocida*: Its variable nature demonstrated by in vivo passages. Avian Dis. 37:781-785. 1993.

75. Melamed, D., G. Leitner, and E. D. Heller. A vaccine against avian colibacillosis based on ultrasonic inactivation of *Escherichia coli*. Avian Dis. 35:17-22. 1991.

76. Moorhead, P. D., and Y. M. Saif. Mycoplasma meleagridis and *Escherichia coli* infections in germ-free and specific-pathogen-free turkey poults: pathologic manifestations. Am. J. Vet. Res. 31:1645-1653. 1970.

77. Morishita, T. Y., K. P. Snipes, and T. E. Carpenter. Serum resistance as an indicator of virulence of *Pasteurella multocida* for turkeys. Avian Dis. 34:888-892. 1990.

78. Morris, J. A., and W. J. Sojka. *Escherichia coli* as a pathogen in animals, in The Virulence of *Escherichia coli*, M. Sussman, ed. Academic Press, London, England, pp. 47-77. 1985.

79. Murakami, S., Y. Okazaki, T. Kazama, T. Suzuki, I. Iwabuchi, and K. Kirioka. A dual infection of *Clostridium perfringens* and *Escherichia coli* in broiler chicks. J. Jpn Vet. Med. Assoc. 42:405-409. 1989.

80. Myers, R. K., and L. H. Arp. Pulmonary clearance and lesions of lung and air sac in passively immunized and unimmunized turkeys following exposure to aerosolized *Escherichia coli*. Avian Dis. 31:622-628. 1987.

81. Nabbut, N. H., and I. H. Khatib. Virulence of *Escherichia coli* strains for chicken embryos. Avian Dis. 22:10-15. 1978.

82. Nagaraja, K. V., D. A. Emery, J. A. Newman, and B. S. Pomeroy. Identification and isolation of somatic pili from pathogenic *Escherichia coli* of turkeys. Am. J. Vet. Res. 44:284-287. 1983.

83. Nagaraja, K. V., D. A. Emery, K. A. Jordan, V. Sivanandan, J. A. Newman, and B.S. Pomeroy. Effect of ammonia on the quantitative clearance of *Escherichia coli* from lungs, air sacs, and livers of turkeys aerosol vaccinated against *Escherichia coli*. Am. J. Vet. Res. 45:392-395. 1984.

84. Nagi, M. S., and W. J. Mathey. Interaction of *Escherichia coli* and Eimeria brunetti in chickens. Avian Dis. 16:864-873. 1972.

85. Nakamura, K., N. Yuasa, H. Abe, and M. Narita. Effect of cyclophosphamide on infections produced by *Escherichia coli* of high and low virulence in chickens. Avian Pathol. 16:237-252. 1987.

86. Nakamura, K., N. Yuasa, H. Abe, and M. Narita. Effect of infectious bursal disease virus on infections produced by *Escherichia coli* of high and low virulence in chickens. Avian Pathol. 19:713-721. 1990.

87. Nakamura, K., M. Maeda, Y. Imada, T. Imada, and K. Sato. Pathology of spontaneous colibacillosis in a broiler flock. Vet. Pathol. 22:592-597. 1985.

88. Nakamura, K., J. K. A. Cook, J. A. Frazier, and M. Narita. *Escherichia coli* multiplication and lesions in the respiratory tract of chickens inoculated with infectious bronchitis virus and/or *E. coli*. Avian Dis. 36:881-890. 1992.

89. Nakamura, K., M. Narita, K. Imai, T. Matsumura, M. Maeda, and T. Tanimura. The effect of mixed live vaccines of Newcastle disease and infectious bronchitis on the chicken respiratory tract. J. Comp. Pathol. 106:341-350. 1992.

90. Naveh, M. W., T. Zusman, E. Skutelsky, and E. Z. Ron. Adherence pili in avian strains of *Escherichia coli*: effect on pathogenicity. Avian Dis. 28:651-661. 1984.

91. Newberry, L. A., J. K. Skeeles, D. L. Kreider, J. N. Beasley, J. D. Story, R. W. McNew, and B. R. Berridge. Use of virulent hemorrhagic enteritis virus for the induction of colibacillosis in turkeys. Avian Dis. 37:1-5. 1993.

92. Nolan, L. K., R. E. Wooley, J. Brown, K. R. Spears, H. W. Dickerson, and M. Dekich. Comparison of a complement resistance test, a chicken embryo lethality test, and the chicken lethality test for determining virulence of avian *Escherichia coli*. Avian Dis. 36:395-397 1992a.

93. Nolan, L. K., R. E. Wooley, and R. K. Cooper. Transposon mutagenesis used to study the role of complement resistance in the virulence of an avian *Escherichia coli* isolate. Avian Dis. 36:398-402. 1992b.

94. Nolan, L. K., R. E. Wooley, C. W. Giddings, and J. Brown. Characterization of an avirulent mutant of a virulent avian *Escherichia coli* isolate. Avian Dis. 38:146-150. 1994.

95. Odagiri, Y., A. E. Jensen, and N. F. Cheville. Immunity of chicks against colibacillosis by vaccination of parent breeders. Bull. Univ. Osaka Pref. [B] 40:37-44. 1988.

96. Piercy, D. W. T., and B. West. Experimental *Escherichia coli* infection in broiler chickens: course of the disease induced by inoculation via the air sac route. J. Comp. Pathol. 86:203-210. 1976.

97. Powell, P. C. Immune mechanisms in infections of poultry. Vet. Immunol. Immunopathol. 15:87-113. 1987.

98. Prantner, M. M., B. G. Harmon, J. R. Glisson, and E. A. Mahaffey. Septicemia in vaccinated and nonvaccinated turkeys inoculated with *Pasteurella multocida* serotype A:3,4. Vet. Pathol. 27:254-260. 1990.

99. Rhoades, K. R., and R. B. Rimler. Fowl cholera, in Pasteurella and Pasteurellosis, eds. Adlam, C., and J.M. Rutter. Academic Press, London, pp. 95-115. 1989.

100. Rhoades, K. R., and R. B. Rimler. *Pasteurella multocida* colonization and invasion in experimentally exposed turkey poults. Avian Dis. 34:381-383. 1990.

101. Rhoades, K. R., and R. B. Rimler. Pasteurellosis, in Diseases of Poultry, eds. B.W. Calnek, H. J. Barnes, C. W. Beard, W. M. Reid, and H. W. Yoder, Jr. Iowa State Univ. Press, Ames, IA, pp. 145-162, 1991.

102. Rhoades, K. R., and R. B. Rimler. *Pasteurella multocida* virulence factors: selection of fowl cholera-inducing and non-inducing strains. Avian Dis. 37:1071-1073, 1993.

103. Rimler, R. B., and K. R. Rhoades. Fowl cholera: influence of *Bordetella avium* on vaccinal immunity of turkeys to *Pasteurella multocida*. Avian Dis. 30:838-839. 1986.

104. Rosenberger, J. K., P. A. Fries, S. S. Cloud, and R. A. Wilson. In vitro and in vivo characterization of avian *Escherichia coli*. II. Factors associated with pathogenicity. Avian Dis. 29:1094-1107. 1985a.

105. Rosenberger, J. K., P. A. Fries, and S. S. Cloud. In vitro and in vivo characterization of avian *Escherichia coli*. III. Immunization. Avian Dis. 29:1108-1117. 1985b.

106. Sacco, R. E., Y. M. Saif, K. E. Nestor, N. B. Anthony, D. A. Emmerson, and R.N. Dearth. Genetic variation in resistance of turkeys to experimental challenge with *Pasteurella multocida*. Avian Dis. 35:950-954. 1991.

107. Saif, Y. M., P. D. Moorehead, and E. H. Bohl. Mycoplasma meleagridis and *Escherichia coli* infections in germfree and specific-pathogen-free turkey poults: production of complicated airsacculitis. Am. J. Vet. Res. 31:1637-1643. 1970.

108. Smith, H. W., J. K. A. Cook, and Z. E. Parsell. The experimental infection of chickens with mixtures of infectious bronchitis virus and *Escherichia coli*. J. Gen. Virol. 66:777-786. 1985.

109. Soerjadi, A. S., S. M. Stehman, G. H. Snoeyenbos, O. M. Weinack, and C. F. Smyser. Some measurements of protection against paratyphoid Salmonella and *Escherichia coli* by competitive exclusion in chickens. Avian Dis. 25:706-712. 1981.

110. Sojka, W. J. *Escherichia coli* in domestic animals and poultry. Commonwealth Agric. Bur., Farnham Royal, England. pp. 157-169. 1965.

111. Springer, W. T., C. Luskus, and S. S. Pourciau. Infectious bronchitis and mixed infections of Mycoplasma synoviae and *Escherichia coli* in gnotobiotic chickens. I. Synergistic role in the airsacculitis syndrome. Infect. Immun. 10:578-589. 1974.

112. Stuart, J. C. Rhinotracheitis: turkey rhinotracheitis (TRT) in Great Britain. In, Recent Advances in Turkey Science, Butterworths, London, pp. 217-224. 1989.

113. Suwanichkul, A., and B. Panigrahy. Biological and immunological characterization of pili of *Escherichia coli* serotypes O1, O2, O78 pathogenic to poultry. Avian Dis. 30:781-787. 1986.

114. Tarwid, J. N., R. J. Cawthorn, and C. Riddell. Cryptosporidiosis in the respiratory tract of turkeys in Saskatchewan. Avian Dis. 29:528-532. 1985.

115. Tengerdy, R. P., and C. F. Nockels. Vitamin E or vitamin A protects chickens against *E. coli* infection. Poult. Sci. 54:1292-1296. 1975.

116. Tengerdy, R. P., and J. C. Brown. Effect of vitamin E and A on humoral immunity and phagocytosis in *E. coli* infected chicken. Poult. Sci. 56:957-962. 1977.

117. Tengerdy, R. P., N. G. Lacetera, and C. F. Nockels. Effect of beta carotene on disease protection and humoral immunity in chickens. Avian Dis. 34:848-854. 1990.

118. Toth, T. E., H. Veit, W. B. Gross, and P. B. Seigel. Cellular defense of the avian respiratory system: protection against *Escherichia coli* airsacculitis by *Pasteurella multocida*-activated respiratory phagocytes. Avian Dis. 32:681-687. 1988.

119. Tsuji, M., and M. Matsumoto. Pathogenesis of fowl cholera: influence of encapsulation on the fate of *Pasteurella multocida* after intravenous inoculation into turkeys. Avian Dis. 33:238-247. 1988.

120. Van Alstine, W. G., and L. H. Arp. Effects of *Bordetella avium* infection on the pulmonary clearance of *Escherichia coli* in turkeys. Am. J. Vet. Res. 48:922-926. 1987a.

121. Van Alstine, W. G., and L. H. Arp. Influence of *Bordetella avium* infection on association of *Escherichia coli* with turkey trachea. Am. J. Vet. Res. 48:1574-1576. 1987b.

122. Vidotto, M. C., E. E. Müller, J. C. de Freitas, A.A. Alfieri, I.G. Guimaraes, and D.S. Santos. Virulence factors of avian *Escherichia coli*. Avian Dis. 34:531-538. 1990.

123. Weinack, O. M., G. H. Snoeyenbos, C. F. Smyser, and A. S. Soerjadi. Competitive exclusion of intestinal colonization of *Escherichia coli* in chicks. Avian Dis. 25:696-705. 1981.

124. Weinack, O. M., G. H. Snoeyenbos, C. F. Smyser, and A. S. Soerjadi. Reciprocal competitive exclusion of Salmonella and *Escherichia coli* by native intestinal microflora of the chicken and turkey. Avian Dis. 26:585-595. 1982.

125. White, D. G., M. Dho-Moulin, R. A. Wilson, and T. S. Whittam. Clonal relationships and variation in virulence among *Escherichia coli* strains of avian origin. Microbial Pathogenesis 14:399-409. 1993.

126. Wooley, R. E., K. R. Spears, J. Brown, L. K. Nolan, and O. J. Fletcher. Relationship of complement resistance and selected virulence factors in pathogenic avian *Escherichia coli*. Avian Dis. 36:679-684. 1992a.

127. Wooley, R. E., K. R. Spears, J. Brown, L. K. Nolan, and M. A. Dekich. Characteristics of conjugative R-plasmids from pathogenic avian *Escherichia coli*. Avian Dis. 36:348-352. 1992b.

128. Wooley, R. E., L. K. Nolan, J. Brown, P. S. Gibbs, C. W. Giddings, and K. S. Turner. Association of K-1 capsule, smooth lipopolysaccharides, traT gene, and colicin V production with complement resistance and virulence of avian *Escherichia coli*. Avian Dis. 37:1092-1096. 1993.

129. Wooley, R. E., L. K. Nolan, J. Brown, P. S. Gibbs, and D. I. Bounous. Phenotypic expression of recombinant plasmids pKT107 and pHK11 in an avirulent avian *Escherichia coli*. Avian Dis. 38:127-134. 1994a.

130. Wooley, R. E., J. Brown, P. S. Gibbs, L. K. Nolan, and K. S. Turner. Effect of normal intestinal flora of chickens on colonization by virulent colicin V-producing, avirulent, and mutant colicin V-producing avian *Escherichia coli*. Avian Dis. 38:141-145. 1994b.

131. Yerushalmi, Z., N. I. Smorodinsky, M. W. Naveh, and E. Z. Ron. Adherence pili of avian strains of *Escherichia coli*. Infect. Immun. 58:1129-1131. 1990.

132. Yoder, H. W., Jr., C. W. Beard, and B. W. Mitchell. Pathogenicity of *Escherichia coli* in aerosols for young chickens. Avian Dis. 33:676-683. 1989.

Current Understanding of Bordetellosis

J. Kirk Skeeles

Bordetellosis in turkeys was first described in 1967 (10) but it did not come to prominence until the mid-1970's. A respiratory disease with signs similar to bordetellosis was reported from North Carolina (5) and from Germany in 1978 (15). The causative agent was misidentified in early reports being called *Bordetella bronchiseptica*, *Bordetella bronchiseptica*-like, avian adenovirus and in 1979 the disease was very well described in turkeys but the causative agent was again misidentified to be *Alcaligenes faecalis* (30,32). In 1984 it was finally correctly identified to be a new Bordetella species, *Bordetella avium* (23).

My first experience with the disease was as a graduate student at the University of Georgia working on infectious bursal disease virus in chickens. I was given bursae from turkeys from North Carolina to attempt to isolate virus. A visit by a veterinarian to a turkey farm in North Carolina where a flock of turkeys was experiencing severe respiratory disease showed these turkeys to have small atrophied bursae which gave rise to the idea of a possible turkey bursal disease virus. A hypothesis was made that there might be an immunosuppressive condition in turkeys as had been described in the chicken. A virus isolation was made from these turkeys but it was found to be nonpathogenic.

Later that same year, 1978, I moved to Arkansas into the middle of an epidemic of severe respiratory disease in turkeys and it was quickly evident that bursal damage was not a feature of the disease. The disease as observed in Arkansas was manifested by high morbidity and mortality. The mortality was caused by colibacillosis. Early clinical signs included snicking, dirty foamy eyes and a clear nasal exudate when the sinuses were depressed. Bacterial cultures of the trachea resulted in the isolation of small smooth mucoid colonies that were approximately 1.0 mm in diameter at 24 hours. These organisms were non-fermentative and gram negative. The disease continued to be a major problem to the turkey industry in Arkansas and Southwest Missouri throughout the early 1980's but with each passing year the disease became noticeably less severe.

The disease has not completely disappeared but it is certainly not the major problem it once was to the turkey industry at least in our area. I think some of the success in controlling the disease can be attributed to better management and disease control practices but I also feel that there are factors concerning the bacteria and possibly the turkey that have also contributed to the decline in importance of this disease.

Clinical signs of *B. avium* infection are depression, sinusitis, foamy eyes and respiratory distress (29,30,31). Signs of infection are most often seen in young birds 2- to 8 weeks post hatch with the upper respiratory tract being the area of involvement in a primary infection. *B. avium* infections can be mild if there are no other complications but clinical disease and mortality can be severe if it occurs in conjunction with other infectious agents, environmental stresses and vaccination reactions. *B. avium* plus other complications can lead to severe colibacillosis (28,35).

The mechanism of pathogenesis of severe *B. avium*-related colibacillosis is probably related to the ability of certain strains of the bacteria to colonize the ciliated tracheal epithelium with resultant deciliation and loss of mucus gland function (2,9,13). There have also been reports of damage to the tracheal cartilage with distortion of the tracheal rings and collapse. Some mortality has been related to suffocation from collapse of the trachea. The damage to the

mucociliary protective apparatus in the trachea undoubtedly opens the gate for *E. coli* (8,27,33,34) with the disease being much more severe when sanitation is poor. I consider *B. avium*, at least, in its old form to be a major initiator of colibacillosis in the turkey.

Important factors involved in the ability of *B. avium* to damage the tracheal epithelium also include adherence factors (1,22) and the ability to produce toxins (12,26). *B. avium* has been shown to produce several toxins some of which appear to be similar to those from other Bordetella sp. (12).

Related factors that appear to effect the severity of the disease include age of infection and the presence of maternal antibody. In our experience the younger birds are infected the more severe the disease with age resistance possibly being a factor. The presence of maternal antibody also has an effect on the severity of the disease (25).

Vaccination has included the development and use of bacterins, a live temperature sensitive mutant vaccine and *B. avium*-like bacteria (3,6,7,11,14,16,18,20,21). These vaccines have given mixed results depending on the age, method of vaccination and the challenge strain of *B. avium* utilized.

Diagnosis of *B. avium* infection is done by isolation and serology. A microagglutination test (19) and ELISA (17) have been developed and widely used. A commercial ELISA test kit will be marketed soon.

B. avium infection has also been reported in chickens but the chicken is apparently much more resistant than the turkey. Reproduction of the disease in the chicken has not been accomplished with any consistency (4,24,28).

A number of reports from the field have associated bordetellosis with stagnant water. This has led to much more attention to disinfecting water lines and waterers. *B. avium* is readily transmitted by direct contact and mechanical transmission (29). This is especially a problem on multiple age farms.

So what has happened to bordetellosis and why is it not the problem at least in Arkansas and southwestern Missouri that it was in the 1970's and early 1980's? I suspect that there are strains of *B. avium* that are now prevalent that do not have the ability to produce the toxins that cause the deciliation and tracheal cartilage damage. Breeders may now be passing maternal antibody that helps prevent colonization of the trachea. More attention is being given to correct the management practices that contributed to the severity of the disease that was once observed.

REFERENCES

1. Arp, L. H., and E. E. Brooks. An in vivo model for the study of *Bordetella avium* adherence to tracheal mucosa in turkeys. Am. J. Vet. Res. 47(12):2614-2617. 1986.

2. Arp, L. H.,and N. F. Cheville. Tracheal lesions in young turkeys infected with *Bordetella avium*. Am. J. Vet. Res. 45(10):2196-2200. 1984.

3. Barnes, H. J., and M. S. Hofstad. Susceptibility of turkey poults from vaccinated and unvaccinated hens to *Alcaligenes rhinotracheitis* (turkey coryza). Avian Dis. 27:378-392. 1983.

4. Berkhoff, H. W., F. M. McCorkle, and T. T. Brown. Pathogenicity of various isolates of *Alcaligenes faecalis* for broilers. Avian Dis. 27(3):707-713. 1983.

5. Blalock, H. G., D. G. Simmons, K. E. Muse, J. G. Gray, and W. T. Derieus. Adenovirus respiratory infection in turkey poults. Avian Dis. 19:707-716. 1975.

6. Burke, D. S., and M. M. Jensen. Immunization against turkey coryza by colonization with mutants of *Alcaligenes faecalis*. Avian Dis. 24:726-733. 1980.

7. Burke, D. S., and M. M. Jensen. Field vaccination trials against turkey coryza using a temperature-sensitive mutant of *Alcaligenes faecalis*. Avian Dis. 25:96-103. 1981.

8. Ficken, M. D., J. F. Edwards, and J. C. Lay. Clearance of bacteria in turkeys with *Bordetella avium*-induced tracheitis. Avian Dis. 30(2):352-357. 1986.

9. Ficken, M. D., J. F. Edwards, J. C. Lay, and D. E. Tveter. Tracheal mucus transport rate in normal turkeys and in turkeys infected with *Bordetella avium* (*Alcaligenes faecalis*). Avian Dis. 30(1):154-159. 1986.

10. Filion, P. R., S. Cloutier, E. R. Vrancken, and G. Bernier. Infection respiratoire du dindonneau causee par un microbe apparente au *Bordetella bronchiseptica*. Can. J. Comp. Med. Vet. Sci. 31:127-134. 1967.

11. Garcia, R. *Alcaligenes faecalis*-laboratory and field studies evaluating the efficacy and safety of a live ART vaccine. Proceedings of the 33rd Western Poultry Disease Conference, Davis, California 24-27. 1984.

12. Gentry-Weeks, C. R., B. T. Cookson, W. E. Goldman, R. B. Rimler, S. B. Porter, and R. Curtiss III. Dermonecrotic toxin and tracheal cytotoxin, putative virulence factors of *Bordetella avium*. Infect. and Immun. 56(7):1698-1707. 1988.

13. Gray, J. G., J. F. Roberts, R. C. Dillman, and D. G. Simmons. Pathogenesis of change in the upper respiratory tracts of turkeys experimentally infected with an *Alcaligenes faecalis* isolate. Infect. and Immun. 42(1):350-355. 1983.

14. Herzog, M., M. F. Slavik, J. K. Skeeles, and J. N. Beasley. The efficacy of a temperature-sensitive mutant vaccine against northwest Arkansas isolates of *Alcaligenes faecalis*. Avian Dis. 30:112-116. 1986.

15. Hinz, K. H., G. Glunder, and H. Luders. Acute respiratory disease in turkey poults caused by *Bordetella bronchiseptica*-like bacteria. Vet. Rec. 103:262-263. 1978.

16. Hofstad, M. S., and E. L. Jeska. Immune response of poults following intranasal inoculation with Art Vax vaccine and a formalin-inactivated *Bordetella avium* bacterin. Avian Dis. 29:746-765. 1985.

17. Hopkins, B. A., J. K. Skeeles, G. E. Houghten, and J. D. Story. Development of an enzyme-linked immunosorbent assay for *Bordetella avium*. Avian Dis. 32:353-361. 1988.

18. Houghten, G. E., J. K. Skeeles, M. Rosenstein, J. N. Beasley, and M. F. Slavik. Efficacy in turkeys of spray vaccination with a temperature-sensitive mutant of *Bordetella avium* (Art Vaxtm). Avian Dis. 31:309-314. 1987.

19. Jackwood, D. J., and Y. M. Saif. Development and use of a microagglutination test to detect antibodies to *Alcaligenes faecalis* in turkeys. Avian Dis. 24:685-701. 1980.

20. Jackwood, M. W., and Y. M. Saif. Efficacy of a commercial turkey coryza vaccine (Art-Vax) in turkey poults. Avian Dis. 29:1130-1139. 1985.

21. Jackwood, M. W., and Y. M. Saif. Lack of protection against *Bordetella avium* in turkey poults exposed to *B. avium*-like bacteria. Avian Dis. 31:597-600. 1987.

22. Jackwood, M. W., and Y. M. Saif. Pili of *Bordetella avium*: expression, characterization, and role in in vitro adherence. Avian Dis. 31(2):277-286. 1987.

23. Kersters, K., K. H. Hinz, A. Hertle, P. Segers, A. Lievens, O. Siegman, and J. Deley. *Bordetella avium* sp. nov. isolated from the respiratory tracts of turkeys and other birds. Int. J. Syst. Bacteriol. 34:56-70. 1984.

24. Montgomery, R. D., S. H. Kleven, and P. Villegas. Observations on the pathogenicity of *Alcaligenes faecalis* in chickens. Avian Dis. 27(3):751-761. 1983.

25. Neighbor, N. K., J. K. Skeeles, J. N. Beasley and D. L. Kreider. Use of an enzyme-linked immunosorbent assay to measure antibody levels in turkey breeder hens eggs, and progeny following natural infection on immunization with a commercial *Bordetella avium* bacterin. Avian Dis. 35:315-320. 1991.

26. Rimler, R. B. Turkey coryza: toxin production by *Bordetella avium*. Avian Dis. 29(4):1043-1047. 1985.

27. Saif, Y. M., P. D. Moorehead, R. N. Dearth, and D. J. Jackwood. Observations on *Alcaligenes faecalis* infection in turkeys. Avian Dis. 24(3):665-684. 1980.

28. Simmons, D. G., D. E. Davis, L. P. Rose, J. G. Gray, and G. H. Luginbuhl. *Alcaligenes faecalis*-associated respiratory disease of chickens. Avian Dis. 25(3):610-613. 1981.

29. Simmons, D. G., and J. G. Gray. Transmission of acute respiratory disease (Rhinotracheitis) of turkeys. Avian Dis. 23(1):132-138. 1979.

30. Simmons, D. G., G. G. Gray, L. P. Rose, R. C. Dillman, and S. E. Miller. Isolation of an etiologic agent of acute respiratory disease (Rhinotracheitis) of turkey poults. Avian Dis. 23(1):194-203. 1979.

31. Simmons, D. G., L. P. Rose, and J. G. Gray. Some physical, biochemical, and pathologic properties of *Alcaligenes faecalis*, the bacterium causing rhinotracheitis (coryza) in turkey poults. Avian Dis. 24(1):82-90. 1980.

32. Slavik, M. F., J. K. Skeeles, J. N. Beasley, G. C. Harris, P. Roblee, and D. Hellwig. Effect of humidity on infection of turkeys with *Alcaligenes faecalis*. Avian Dis. 25(4):936-942. 1981.

33. Van-Alstine, W. G., and L. H. Arp. Effects of *Bordetella avium* infection on the pulmonary clearance of *Escherichia coli* in turkeys. Am. J. Vet. Res. 48(6):922-926. 1987.

34. Van-Alstine, W. G., and L. H. Arp. Influence of *Bordetella avium* infection on association of *Escherichia coli* with turkey trachea. Am. J. Vet. Res. 48(11):1574-1576. 1987.

35. Yegana, Y., Y. Weisman, A. Herz, R. Schapira, and I. Hod. The effects of combined and separate infection by *Alcaligenes faecalis* and paramyxovirus (Yucaipa) on the surface morphology of the trachea in turkey poults. Avian Path. 14:543-551. 1985.

Infectious Coryza: An Emerging Disease in Broiler Production

John R. Glisson

The impression among poultry veterinarians within the U. S. is that the incidence of infectious coryza in the broiler industry is increasing. This impression has been strengthened by several fairly dramatic cases in broilers and broiler breeders in the last two years in the southeastern U. S. For the past decade or longer, infectious coryza has been a rare disease in the broiler industry so these recent cases have fueled a fear that this once-forgotten problem may have to be dealt with again.

It is difficult to determine precisely if the incidence of coryza has increased from the available data (Tables 1, 2). It is clear that there has been no dramatic increase in reported cases of infectious coryza, however, several states do not report poultry disease diagnoses and the data from other states may represent only a portion of the cases seen. A case may represent several farms within one company and be reported as a single case. The impression of field poultry veterinarians is likely to be more accurate than the disease reporting system and there truly has been an increased incidence of infectious coryza in the broiler industry.

We have been involved directly in the diagnosis of a few cases and peripherally in several others. It has not always been possible to determine a plausible avenue of entry of the Hemophilus organism onto the farms in every case. In some cases, the use of migrant or alien labor has been incriminated because these people are considered likely to contact backyard chickens and fighting cocks. Other cases occurred in commercial flocks in close proximity to backyard poultry. In general, most cases appear to be traceable to a lack of strict biosecurity.

Many cases have involved very high mortality and condemnation in broilers or high morbidity and severe egg production drops in broiler breeders. This should signal that the causative organism, *Haemophilus paragallinarum*, still exists in commercial poultry rearing regions and that strict biosecurity is needed to prevent its entry into commercial chickens.

Table 1. Infectious Coryza Cases Reported in North America[a].

		Region				
Category	Year	NC	NE	SO	WE	CAN
Broiler	90	-	-	1	-	-
Layer	90	-	-	6	-	-
Other	90	-	-	4	-	-
Broiler	91	-	-	12	-	-
Layer	91	-	-	9	-	-
Other	91	-	-	3	-	-
Broiler	92	-	1	4	-	-
Layer	92	-	-	7	-	-
Other	92	-	1	4	-	-

[a]National Poultry Disease Incidence Report.

Table 2: Infectious Coryza Cases Reported in Southern Region of the U.S.A., 1993[a].

	State						
Category	AL	AR	FL	GA	SC	TX	NC
Broiler	-	-	1	1	1	1	-
Layer	-	-	-	3	-	-	-
Other	-	2	-	-	-	-	-

[a]Southern Conference of Avian Diseases Report, 1993.

Environmental Respiratory Pathogens: Poultry House Gases and Dust

Jean E. Sander

The environment in a poultry house is a combination of many factors interacting in a complex, dynamic system. Birds are exposed continuously to aerial pollutants in the form of organic and inorganic dusts, microbes, endotoxins, and noxious gases (3,11,18). These atmospheric pollutants concentrate in poultry houses during winter months when ventilation is at a minimum (3).

Spacial and temporal distribution of aerial pollutants in large livestock buildings are determined mainly by airflow patterns and rates of air exchange, method and frequency of feeding, type of bedding and manure removal, physical layout of the building and its internal structures, and activity of the animals (11). Current design and maintenance of ventilation systems for animal confinement housing results in unhealthy levels of dust, gases, and microbes (27). Flock health and productivity may be harmed by chronic exposure to these burdens, especially in the presence of simultaneous challenge by respiratory pathogens (11). There is a strong association between respiratory diseases and air quality in animal confinement units (27).

GASES

Potential problem gases occurring in confinement poultry houses include ammonia, hydrogen sulfide, methane, carbon dioxide, carbon monoxide, and nitrogen oxides (15, 23). Exposure to toxic gases appears to be related to both concentration and length of exposure (4). Under normal circumstances carbon dioxide and hydrogen sulfide would not reach concentrations that would likely create problems (23,28). Ammonia is by far the most common irritant gas affecting poultry health and productivity. Irritant gases are not restricted to the confinement rearing house, however. Chicks may be exposed to formaldehyde gas used as fumigant to control hatchery contamination.

Ammonia. Ammonia is generated in poultry houses by microbial degradation of poultry waste (21,23). It is a product of bacterial deamination or a reduction of the nitrogenous fraction of manure (4). Ammonia is a highly irritating gas that accumulates to high levels with increases in litter moisture, pH, temperature, aeration, and fecal matter content (3,24,30). Ammonia levels increase steadily over time in a populated confinement facility. Reuse of litter material is directly related to ammonia levels and associated disease conditions (11,25). These include keratoconjunctivitis, dermatitis, bursal atrophy, airsacculitis, and colibacillosis (4,9,21,22,24,25).

The respiratory tract lining is normally covered with aqueous mucus. Therefore, gases such as ammonia, which are highly soluble in water, are absorbed from the inspired air by the upper respiratory mucous. Ammonia rarely reaches the lungs but gases can be absorbed and carried deeper on aerial dust (13). Dust and ammonia seem to have a synergistic effect (27). Ammoniated alveolar air gives rise to ammonia by-products in the blood. This may alter blood pH resulting in decreased respiratory activity. Exposure to 78 ppm of ammonia for 15 minutes did not significantly alter blood pH, however (9).

Ammonia affects birds by causing physical damage to the respiratory tract. Nagaraja demonstrated excessive mucous production, matted cilia, and areas of deciliation in the tracheas of birds exposed to ammonia concentrations as low as 10 μl/L (31). The damage to the respiratory tract is not always detectable, especially if the irritant gas is at low levels, but they may cause a problem when the birds are exposed to infectious agents (24).

Irritant gases affect the rate of mucous flow or ciliary activity. Exposure to small amounts of irritant gas for long periods of time may cause chronic irritation of the mucosa and affect ciliary activity. The thickness of the mucous blanket is primarily dependent on the activity of the secretory mechanism and mucous glands. The depth of the mucous blanket exerts a direct influence on the power of the cilia by increasing the mechanical pressure. Secretory streaming (the flow rate of of mucus) is estimated to be insignificant at a distance from the cilia 4- to 5 times their length. Altered composition of the mucus also affects the velocity of mucus flow. An increase in the amount of mucin in mucus will increase the viscosity and decrease the rate of flow (14).

Infection occurs more rapidly if the mucociliary blanket is impaired (24). Anderson found that exposure to 20- to 50 ppm of ammonia for 72 hours increased the infection rate of chickens exposed to aerosol of Newcastle disease virus (1). Nagaraja exposed turkeys to an aerosol of *Escherichia coli* with 0- to 40 ppm of ammonia and found *E. coli* in the lungs of birds exposed to ammonia (32).

Exposure to continuous ammonia as low as 20 ppm may result in slight lacrimation and eye discomfort, anorexia, and subsequent weight loss (3). However, at these levels little pathology and no discomfort was detected before 6 weeks of exposure time (1). At levels of 200 ppm discomfort was noted within a few days but the ocular irritation resolved by day 17. Ammonia at 1000 ppm caused photophobia and lacrimation within 3 days. By the 8th day corneal opacities and surface erosions were present (4). An increase in breast blisters and severity of coccidiosis has also been reported (5).

Ammonia has also been reported to have an adverse effect on productivity. It is reported that ammonia can delay sexual maturity causing birds to come into lay later, lay larger but fewer eggs, and increases mortality (5). Any factor that affects feed intake leads to a detrimental effect on mature body weight and ovarian development (16). However, levels of 100 ppm can be tolerated by leghorn hens for short periods of time without drastic losses in laying performance (17).

Fifty ppm of ammonia reduces feed efficiency from 1- to 49 days of age in broilers (5). Removal of ammonia at 29 days, however, improves feed efficiency (8). A level of 106 ppm decreases feed consumption by 14.5% in broilers over 28 days of age. In Leghorns over 15 weeks of age, the decrease in feed consumption was dose related to the ammonia levels (9).

Formaldehyde. Formaldehyde acts as an irritant to eyes and the upper respiratory tract (12). Formaldehyde is highly soluble in water; 50 times more soluble than ammonia. Contact of formaldehyde with the mucous in the trachea produces a shift in pH towards acidity (14). The acidic environment adversely affects ciliary activity causing rapid cessation of movement. The effect is dose related. As the concentration of formaldehyde increases cell death and proliferation increases and mucociliary activity decreases (12).

Eggs exposed to vaporized formaldehyde in the hatcher resulted in chicks with damaged epithelia, deciliation, flattened cells, inflammation, hemorrhage, cell proliferation, and missing mucous cells. The damage was restricted to the upper part of the trachea and remained for 5- to 12 days (19). The author (Sander, *et al.*, unpublished work) has found blunting and

blebbing occurring in the tracheal cilia of chicks exposed to formaldehyde in the hatcher during pipping. Mucus was increased in chicks 5 days after exposure to formaldehyde fumigation.

DUST

The composition and size of the aerosolized dust found in livestock buildings depends on the type and method of feeding, type of floor and bedding material, and the species and activity of the animal (2,15). Poultry house dust contains primarily organic material (6) and is 92% dry matter of which 6% is crude protein, 9% fat, 4% cellulose, and > 4% ash and hydrocarbons (15). It consists of broken feather barbules, skin debris, feed particles, litter components, and microorganisms (3). Dust generation rates vary from 2.5- to 90 mg/hour/bird (6).

Particle size is important in how it affects the respiratory system. Particles > 10 μm deposit in the nasal passages and can cause nasal blockage (26), those between 5- and 10 μm are retained in the upper respiratory tract, and particles < 5 μm are able to reach the air sacs and lungs (6). Ninety percent of the dust in poultry houses is 1- to 5 μm in diameter (20). The majority of particles are < 1 μm in diameter but most of the mass is from particles > 3 μm. Therefore, a predominance of the particles are capable of invading a host through the respiratory tract (15). Respirable particles can irritate the lower airways and by overwhelming the lung clearance mechanisms may increase susceptibility to infection by pathogenic organisms. In addition to causing damage by macrophage blockade, exposure to organic dust can sensitize the lung and may lead to hypersensitivity reactions (26).

One of the major poultry health problems is airsacculitis, an avian respiratory disease that can be caused by a variety of infectious bacterial and fungal organisms growing in the birds lungs and air sacs (30). There is a direct linear correlation between dust levels and airsac lesions in turkeys. The incidence of airsacculitis doubled when dust levels increased from 0.1 - 0.4 to 0.6 - 1.0 mg/ft^3 of dust (3). Infectious process condemnations are related to air sac disease (29). Suspended particulate matter at levels of 1.16 mg/ft^3 have been reported (3).

Dust has not been found to have an adverse effect on resistance to Newcastle disease virus. It also has not affected feed conversion, body weight, and mortality. Histologic changes have been reported in lungs after exposure to dusty environments (7).

Pathogens. As airborne dust increases, airborne levels of pathogens also increase (23). Viruses, bacteria, and fungi exist attached to inert particles (6). Microorganisms have been measured at 4 X 10^5 viable particles/ft^3 (2). Levels of bacteria and fungi average 1.5 X 10^5 and 1.0 X 10^4 CFU/m^3, respectively (23). Bacteria, commonly 1- to 2 μm in diameter, are normally associated with inert particles from 10- to 20 μm in diameter (6,23). In poultry houses 40% of the gram negative bacteria were found on respirable particles (< 5 μm) (10). Newcastle disease virus has been detected on particles >6 μm (39%) and 3- to 6 μm (40%), respectively (6).

Endotoxins. Endotoxins are reported to be the causative agent for human diseases such as found in cotton workers. Clinical signs include cough, headache, nausea, chest tightness, diarrhea, and fever (23). The concentration of airborne endotoxins from gram negative bacteria in poultry units is in a range from 0.12- to 0.5 μg/m^3 with an average concentration of 0.31 μg/m^3. These concentrations can reach as high as 2- to 7 μg/m^3 during the manual catching of chickens (10). The average level of endotoxin has been found to be 6.4- to 16 ng/mg of dust. Endotoxin was detected in all size ranges with the highest concentration of endotoxin per unit of dust found in the smallest size fraction (<3.5 μm) (23).

REFERENCES

1. Anderson, D. P., C. W. Beard, and R. P. Hanson. Influence of poultry house dust, ammonia, and carbon dioxide on the resistance of chickens to Newcastle disease virus. Avian Dis. 10:177-188. 1966.

2. Anderson, D. P., F.L. Cherms, and R. P. Hanson. Studies on measuring the environment of turkeys raised in confinement. Poultry Sci. 43:305-318. 1964.

3. Anderson, D. P., R. R. Wolfe, F. L. Cherms, and M. S. Roper. Influence of dust and ammonia on the development of air sac lesions in turkeys. Am. J. Vet. Res. 29:1049-1058. 1968.

4. Anderson, D. P., C. W. Beard, and R. P. Hanson. The adverse effects of ammonia on chickens including resistance to infection with Newcastle disease virus. Avian Dis. 8:369-379. 1964.

5. Carlile, F. S. Ammonia in Poultry Houses: A literature review. World's Poultry Sci. 40:99-113. 1984.

6. Carpenter, G. A. Dust in livestock buildings - Review of some aspects. J. Ag. Engng. Res. 33:227-241. 1986.

7. Carpenter, G. A., W. K. Smith, A. P. C. MacLaren, and D. Spackman. Effect of internal air filtration on the performance of broilers and the aerial concentrations of dust and bacteria. Brit. Poultry Sci. 27:471-480. 1986.

8. Caveny, D. D. and C. L. Quarles. The effect of atmospheric ammonia stress on broiler performance and carcass quality.Poultry Sci. (Abstract). 57:1124-1125. 1978.

9. Charles, D. R. and C. G. Payne. The influence of graded levels of atmospheric ammonia on chickens. 1. Effects on respiration and on the performance of broilers and replacement growingstock. Brit. Poultry Sci. 7:177-187. 1966.

10. Clark, S., R. Rylander, and L. Larsson. Airborne bacteria, endotoxin and fungi in dust in poultry and swine confinement buildings. Am. Ind. Hyg. Assoc. J. 44:537-541. 1983.

11. Conceicao, M. A. P., H. E. Johnson, and C. M. Wathes. Air hygiene in a pullet house:spatial homogeneity of aerial pollutants. Brit. Poultry Sci. 30:765-776. 1989.

12. Council on Scientific Affairs. Formaldehyde. J.A.M.A. 261:1183-1187. 1989.

13. Curtis, S. E., C. R. Anderson, J. Simon, A. H. Jensen, D. L. Day, and K. W. Kelly. Effects of aerial ammonia, hydrogen sulfide and swine-house dust on rate of gain and respiratory-tract structure in swine. J. Anim. Sci. 41:735-739. 1975.

14. Dalhamn, T. Mucous flow and ciliary activity in the trachea of healthy rats and rats exposed to respiratory irritant gases. Acta Physiol. Scand. 36:76-161. 1956.

15. Dawson, J. R. Minimizing dust in livestock buildings: Possible alternatives to mechanical separation. J. Ag. Engng. Res. 47:235-248. 1990.

16. Deaton, J. W., F. N. Reese, and B. D. Lott. Effect of atmospheric ammonia on pullets at point of lay. Poultry Sci. 63:384-385. 1984.

17. Deaton, J. W., F. N. Reese, and B. D. Lott. Effect of atmospheric ammonia on laying hen performance. Poultry Sci. 61:1815-1817. 1982.

18. Drummond, J. G., S. E. Curtis, and J. Simon. Effects of atmospheric ammonia on pulmonary bacterial clearance in the young pig. Am. J. Vet. Res. 39:211-212. 1978.

19. Gerrits, A. R, and D. J. Dijik. How hatching chicks react when inhaling formalin. Misset-World Poultry 7:17. 1991.

20. Hauser, R. H., and D. W. Folsch. Methods for measuring atmospheric dust, micro-organisms and ammonia in poultry houses: Review and approved practices. J. Vet. Med. 35:579-593. 1988.

21. Huff, W. E., G. W. Malone, and G. W. Chaloupka. Effect of litter treatment on broiler performance and certain litterquality parameters. Poultry Sci. 63:2167-2171. 1984.

22. Johnson, Z. B., R. P. Rorle, D. W. Cross, J. N. Beasley, and G. C. Harris, Jr. Factors influencing incidence of dermatitis in broiler flocks. Ark. Farm Res. 39:4. 1990.

23. Jones, W., K. Morring, S. A. Olenchock, T. Williams, and J. Hickey. Environmental study of poultry confinement buildings. Am. Ind. Hyg. Assoc. J. 45:760-766. 1984.

24. Kling, H. L., and C. L. Quarles. Effect of atmospheric ammonia and the stress of infectious bronchitis vaccination on Leghorn males. Poultry Sci. 53:1161-1167. 1974.

25. Leonard, J. J., J. J. R. Feddes and J. B. McQuitty. Air quality in commercial broiler housing. Can. Ag. Engng. 26:65-71. 1984.

26. Madelin, T. M., and C. M. Wathes. Air hygiene in a broiler house: comparison of deep litter with raised netting floors. Brit. Poult. Sci. 30:23-37. 1989.

27. Maghirang, R. G., H. B. Manbeck, and V. M. Puri. Numerical study of air contaminant distribution in livestock buildings. Am. Soc. Ag. Engng. 34:2171-2180. 1991.

28. McQuitty, J. B., J. J. R. Feddes, and J. J. Leonard. Air quality in commercial laying barns. Can. Ag. Engng. 27:13-19.1985.

29. Morris, M. P. Cellulitis in broilers. Broiler Ind. 54:32-40. 1991.

30. Mulhausen, J. R., C. E. McJilton, P. T. Redig, and K. A. Janni. Aspergillus and other human respiratory disease agents in turkey confinement houses. Am. Ind. Hyg. Assoc. J. 48:894-899. 1987.

31. Nagaraja, K. V., D. A. Emery, K. A. Jordan, J. A. Newman, and B. S. Pomeroy. Scanning electron microscopic studies of adverse effects of ammonia on tracheal tissues of turkeys. Am. J. Res. 44:1530-1536. 1983.

32. Nagaraja, K. V., D. A. Emery, K. A. Jordan, V. Sivanandan, J. A. Newman, and B. S. Pomeroy. Effect of ammonia on the quantitative clearance of *Escherichia coli* from lungs, airsacs, and livers of turkeys aerosol vaccinated against *Escherichia coli*. Am. J. Vet. Res. 45:392-395. 1984.

Economic Aspects of Respiratory Disease in Poultry Meat Production

Calvin E. Anthony

A. Introduction

B. Problem Definition

1. Scope and severity
2. Laboratory diagnosis and drug sensitivities.
3. Age to market
4. General history
    a. Season
    b. Vaccine and vaccination
    c. Farm
    d. Complicating factors

C. Corrective actions or alternative strategies

1. Vaccine
2. Medication
3. Process
4. Destruction

D. Costs of interest

1. Costs of condemnation
2. Consts of medicaton
3. Impact on processing plant and sales of products
4. Other insidious costs of respiratory disease

E. Concluding remarks

The Influence of Breeder and Hatchery Management on Respiratory Disease

Donna Hill

Respiratory disease, expressed as airsacculitis, mortality and condemnations, is a continuing problem in broiler production. There are many different factors that play a role in the incidence and manifestation of respiratory disease. In addition to broiler house management, hatchery and breeder flock management are also important in influencing the health status of a production flock.

The quality of chicks received is a major factor influencing the ability of the production flock to respond to and clear both vaccine and wild respiratory disease pathogens. Chicks that are hatched with yolk sac infections or poorly healed navels have a higher prevalence of bacterial infections. In these chicks the additional stress of vaccination may precipitate the development of bacterial pericarditis, perihepatitis, and airsacculitis. Also, in debilitated chicks vaccine viruses may complete more replication cycles resulting in increasing pathogenicity and a rolling vaccine reaction in the flock.

Chick quality, as it relates to respiratory integrity, is compromised by the following conditions.

1. **Yolk sac infection**

    a. **Egg handling, from the point of lay to the point of hatch.** Eggs for hatching must be properly cooled after being laid and then kept at a proper temperature and humidity throughout the holding and incubation periods. If the eggs are exposed to variable temperatures, the rate of development will be impacted, variable hatch times will result and chick quality will be compromised.

    b. **Sanitation on the farm and in the hatchery.** Dirty egg shells can be a source of bacteria causing yolk sac infections in chicks. To prevent dirty eggs, good nest management and egg collection practices must be followed. In the hatchery, sanitation practices must be consistent and effective, especially in the hatcher and hatcher trays.

    c. **Preventing condensation on eggs during transportation.** Any time moisture condenses on the egg shell, commonly referred to as "sweating", a conduit is established which facilitates bacterial or fungal entry into the egg.

    d. ***E. coli* infection transmitted transovarially from the breeder flock.**

    e. **Wetting of egg shells in the hatchery or breeder house.** Use of fogging nozzles are often times a culprit in both areas.

2. **Poor navel closure.**

   a. **Hatchery air flow and ventilation impacts consistent navel closure.** When air flow is insufficient to meet the hatcher or setter demands, hot spots are created within the machine. Chicks hatched in these areas will have button navels that are a conduit for bacterial challenge to the chick.

   b. **Hatcher and setter operating in unsatisfactory environment.** The more work that a machine must expend to reach set points, the greater the impact on air flow within the machine. Again, uneven air flow creates uneven temperatures and humidities that detrimentally impact chick quality. The environment of the hatchery should be designed to minimize the work load on the setters and hatchers.

   c. **Maintenance of hatcher and setter.** Routine maintenance of the hatcher and setter is critical to assume a consistent and proper air flow within the machines in order to maintain uniform temperature and humidity.

   d. **Inadequate moisture loss or improper dry down of the eggs causes a mushy-bellied chick with a poorly healed navel.** This can be caused by high humidity in the environment and is associated with chicks from older breeder flocks. It can also result from improper wet bulb settings in the hatcher and setter. If enough water does not escape from the egg, the hatching chick may have a large fluid filled yolk sac and an improperly healed navel.

3. **Aspergillosis**. Chicks hatched with aspergillosis have an increased incidence of airsacculitis that can be further aggravated by vaccination. Aspergillus organisms present in the nest can contaminate the eggshell and then penetrate the eggshell as it cools. These contaminated eggs are then brought into the hatchery where the organism is incubated along with the egg. Aspergillus introduced into the hatchery in this way can become established and serve as a continual source of infective organisms for hatching chicks.

4. **Respiratory Stress During Hatch.** Chicks hatched in hatchers with decreased air flow will experience respiratory distress in the hatcher. The actual impact on the chick can vary from transient respiratory distress to hydropericardium. Odom (1) has shown that an increased incidence of ascites in young broiler chicks may be related to an adaptive hypertrophy resulting from early cardiac stress before or just after hatching. Maxwell (2) has found that experimental reduction in the conductance of the egg shell during incubation produces histological lesions in the heart of day-old chicks similar to those seen in chickens exhibiting ascites syndrome. Odom repeated the Maxwell studies and found that these chicks were more susceptible to the development of ascites syndrome, especially if other predisposing factors are present.

Balancing maternal antibodies and the day-of-age vaccination program is critical to maintaining respiratory health in broilers. Consistency of vaccine titer and maternal antibodies is necessary for a successful day-of-age vaccination program.

1. **Newcastle disease virus (NDV) vaccination.**

    a. **Since replication of the virus in the chick is necessary for a protective immune response, the presence of maternal antibodies to Newcastle disease will interfere with the response to day-of-age NDV vaccination.** Chicks with high maternal antibodies are, in effect, not vaccinated at day-of-age and will be exposed later to the vaccine virus via their flock mates. This bird-to-bird transmission increases the virus pathogenicity and contributes to an extended rolling vaccine reaction.

    b. **In chicks with low maternal antibodies, reaction to day-of-age vaccination can be more severe.**

2. **Infectious bronchitis virus vaccination.**

    a. **Since the primary protection against IBV is local, maternal antibodies do not interfere with day-of-age vaccination and it's efficacy.**

    b. **Maternal antibodies can impact the day-of-age vaccine reaction.** The lower the maternal antibodies the greater the vaccine reaction but also the greater the immunity that results.

3. **Proper application of day-of-age vaccination.** This is critical. If vaccination application is not uniform, the chicks that were not vaccinated serve as a host for back passage of the virus which can result in increasing pathogenicity of the vaccine virus.

Vertical transmission of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* through the egg will increase the incidence of respiratory disease in the chicks. The actual impact of these agents will depend on the strain of mycoplasma present, the vaccination program, chick quality, and other diseases present.

To maintain an effective respiratory disease control program in broilers, the program must involve all areas of production, including the breeder flocks and hatcheries. Consistent application of known basics in breeder house and hatchery management can go a long way in preventing respiratory diseases in broilers.

REFERENCES

1. Odom, T. W. Overview and update of ascites syndrome in broilers. Proc. Delmarva Flock Supervisor's Conference, Delmar, Delaware. pp. 30-38. 1992.

2. Maxwell, M. H., S. G. Tullett, and F. G. Burton. Hematological and morphological changes in young broiler chicks with experimentally induced hypoxia. Res. Vet. Sci. 43:331-338. 1987.

Current Management for Prevention and Control of Respiratory Disease in Broilers

Leonard W. Fussell

Management for prevention and control of respiratory disease in broilers is relatively simple, but often complicated by other management diseases. First, the breeder vaccination program plays a key role in achieving success. Our company philosophy is to achieve the following via the breeder immunization program for benefit of the broiler progeny.

* Hyperimmunize for important infectious bursal disease viruses and reoviruses
* Hypoimmunize for Newcastle disease viruses.

Obviously, there are other parts of the breeder immunization program that benefit the progeny directly and indirectly. However, details are deleted here, as that is not the thrust of the assigned topic.

Success in control of respiratory disease and obtaining satisfactory condemnation and performance levels in the production flock is contingent on the following partial list of practices extending from hatch through processing.

* Vaccination for Newcastle disease and infectious bronchitis at one day of age.
* Assure adequate time between flocks in growout houses (layout time).
* Maintain excellent management during early brooding period.
* Revaccination for Newcastle disease and infectious bronchitis to boost immunity. Accurate delivery of vaccine to birds in this field vaccination setting is critical.
* Maintain excellent ventilation/temperature/litter management throughout growout.
* Use nipple drinkers.
* Have a salvage program for birds with air sacculitis in the processing plant.

Average farm size has grown in many regions. This has resulted in a tendency to design housing that is more convenient and labor saving for the grower, but sometimes these modifications result in increased production cost resulting from increased incidence of respiratory disease. Good early brooding and ventilation management practices are critical to an effective vaccination program and achieving satisfactory broiler performance.

Layout time (time between flocks) should average about 14 days. This recommendation is supported by many seasoned veterinarians in the broiler industry and by Agristats data (Table 1; reprinted with permission of Jim Cox).

Nipple drinkers have had the most dramatic impact on respiratory disease in recent years. The data clearly show improvement in livability, feed conversion and condemnation rates (Fig. 1,2,3,4). Similar improvements in health status have been seen in many complexes switching to nipple drinkers throughout the industry.

Table 1. The influence of days between flocks (layout time) on broiler performance.

Performance Criteria	Days between flocks[1]			
	Over 15	12-14.99	9-11.99	Under 9.0
Days to 4 lbs.	44.16	45.24	44.65	45.19
Calorie Conversion (calories/lb body weight)	2833	2882	2910	2936
Livability (%)	94.74	94.62	94.35	94.13
Condemnation (%)	0.992	1.129	1.220	1.429

[1]Agristats special report. The data represent 1,408,020,336 broilers.

The value of boosting chick immunity against Newcastle disease and infectious bronchitis with vaccination during grow out is well recognized. However, proper delivery of the vaccine to the birds is often overlooked. Company personnel or a vaccination crew should do all field vaccinating. Personal preference is spray vaccination with 2-3 people doing the vaccination in each house. It is best to vaccinate birds while they are in the half-house chamber. This practice insures more complete coverage while bird density is high (birds are in half the square footage vs. full house).

Proper litter and ventilation management is important in preventing respiratory diseases. This is an area where service-staff and growers must be diligent. There are many innovations to better manage ventilation and litter in old and new housing, including air cannons, mixing fans, automatic curtain machines, computerized systems, curtain dropper, and attentive growers!

Finally, losses due to respiratory disease can be reduced by implementation of a salvage program for birds with airsacculitis. A program to lower airsacculitis condemnation numbers requires close cooperation between plant personnel, USDA veterinarians, lay inspectors, and company veterinarians and must follow USDA regulations. Since salvage may involve some loss in efficiency (birds per man-hour), the limits of the plant, plant personnel, and USDA concerns are integral in designing a salvage program.

Control of respiratory disease in broilers is paying attention to and implementing these fundamentals. Good luck in your effort.

Figure 1. Body Weight of Straight-Run Broilers Raised with Nipple Drinkers (W/ND) and without Nipple Drinkers (W/O ND).

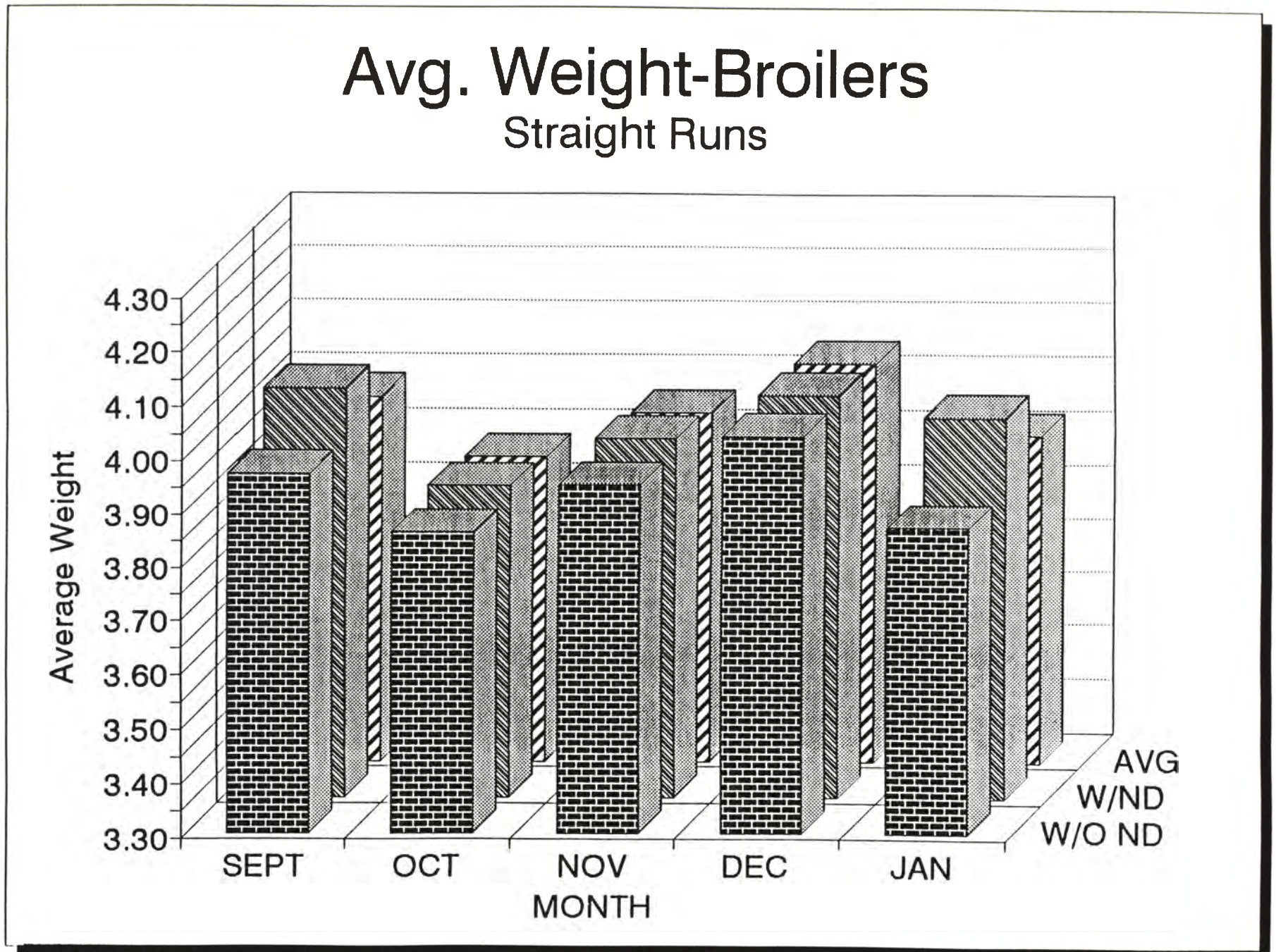


Figure 2. Livability of Straight-Run Broilers Raised with Nipple Drinkers (W/ND) and without Nipple Drinkers (WO/ND).

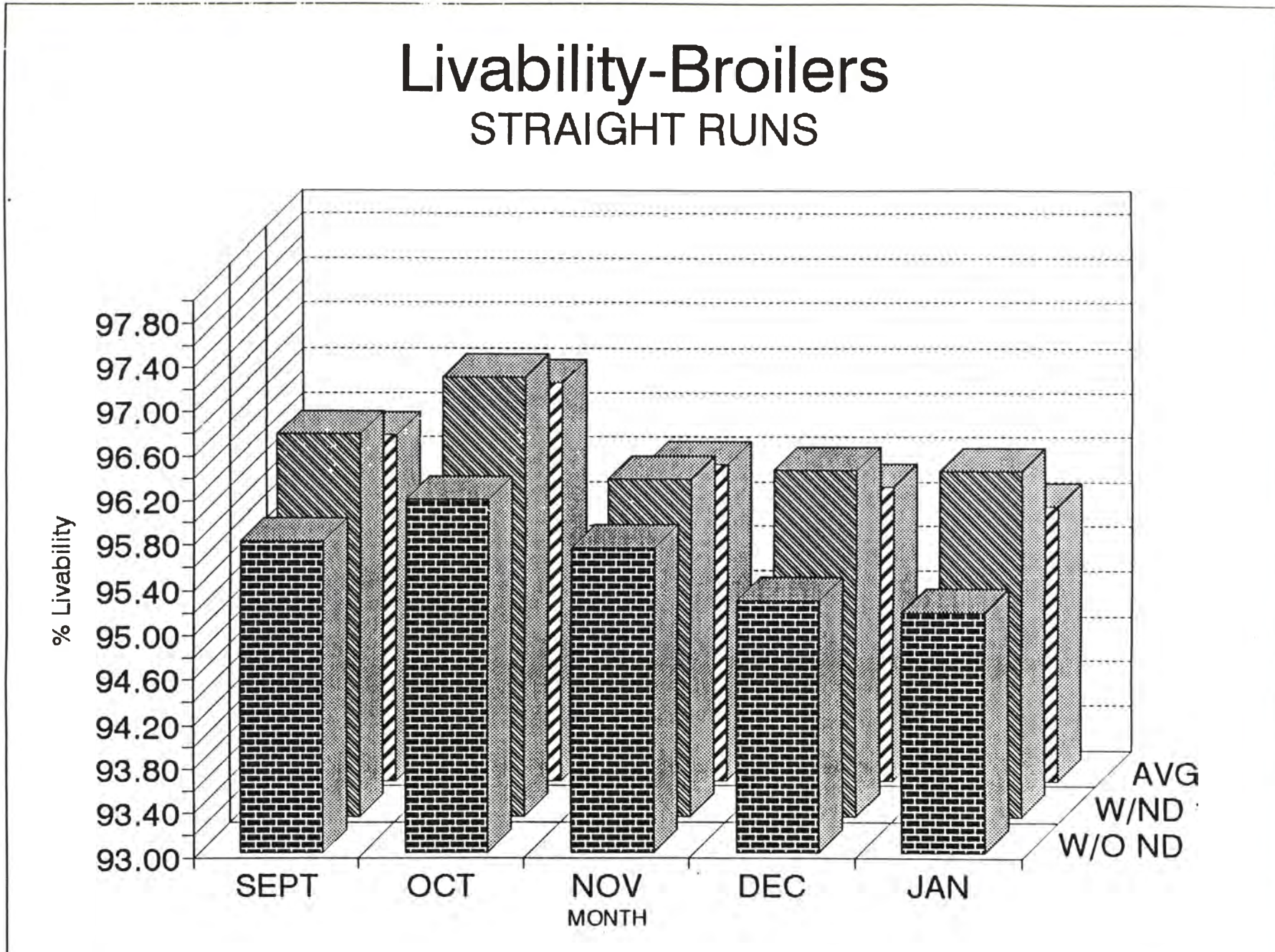


Figure 3. Feed Conversion of Straight-Run Broilers Raised with Nipple Drinkers (W/ND) and without Nipple Drinkers (WO/ND).

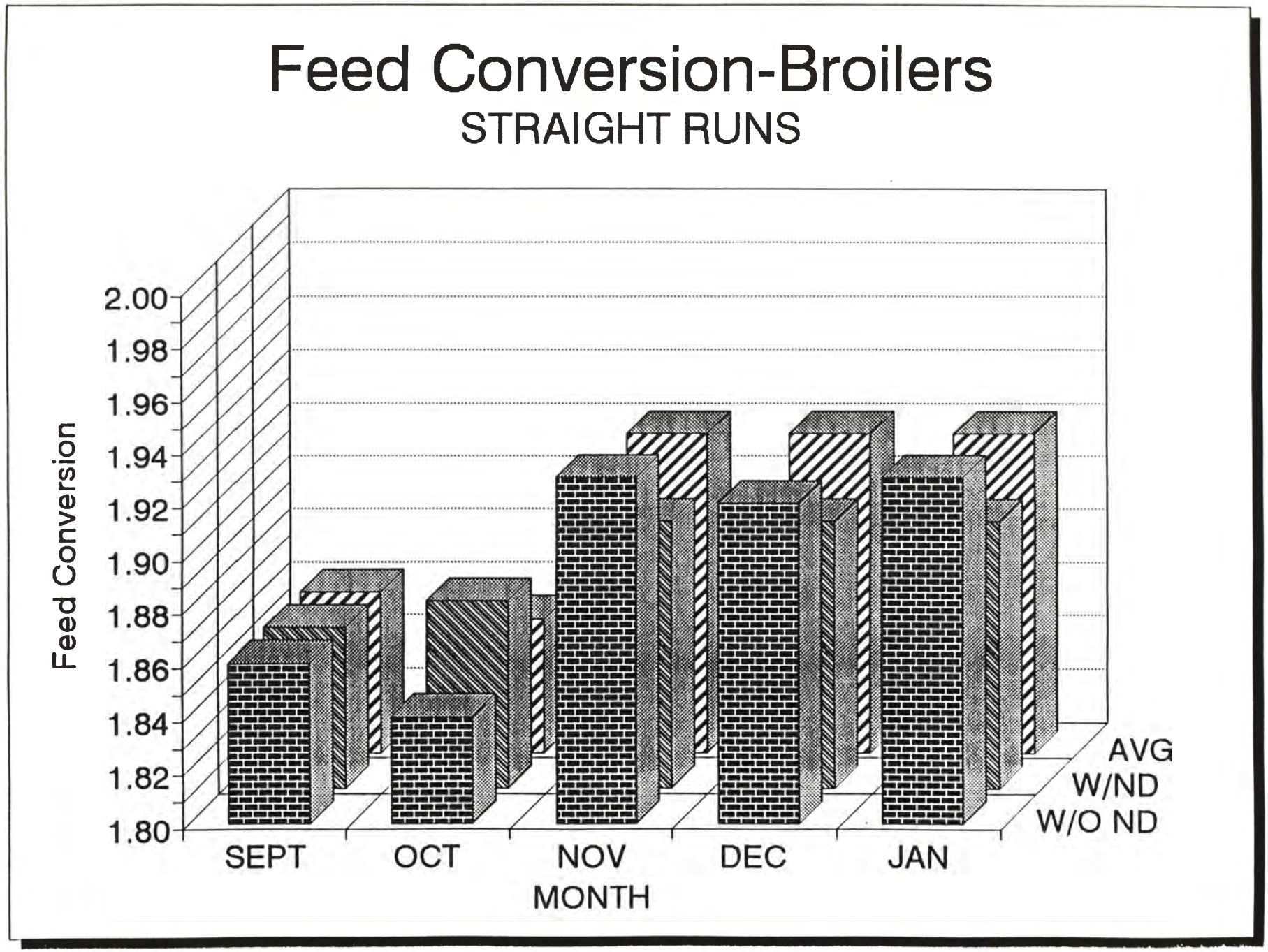
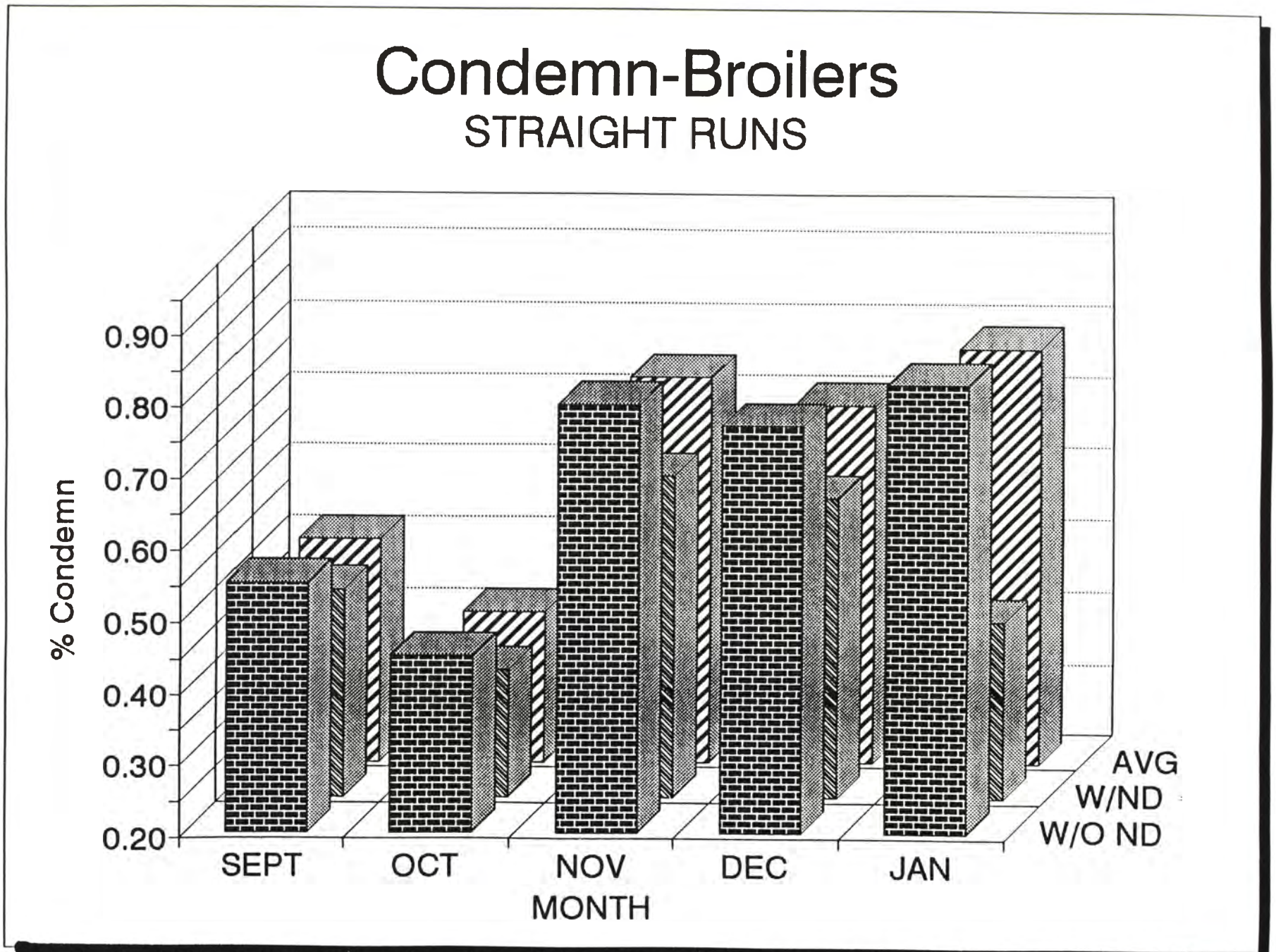


Figure 4. Processing Condemnations of Straight-Run Broilers Raised with Nipple Drinkers (W/ND) and without Nipple Drinkers (WO/ND).



Current Management for the Prevention and Control of Respiratory Disease in Turkeys

Peter E. Poss

BREEDERS

Prevention and control of respiratory disease starts with the basic (primary) breeder and continues through the multiplier breeder operation. Selection of meat-type birds for faster growth and improved conformation historically resulted in cardiopulmonary problems in turkeys. This has been reversed in the past 4 to 5 years by selecting for later maturity which allows skeletal growth to precede muscular development.

Mycoplasma gallisepticum has been eradicated through the National Poultry Improvement Plan (NPIP) breeder candidate testing program. The NPIP has helped to remove *Mycoplasma synoviae* and *Mycoplasma meleagridis* infections from the system, however, they still occur sporadically. Dipping of eggs in antibiotics is used to control the infections in progeny.

Management of egg sanitation and control of bacterial contamination on the farm includes cleaning and disinfection (C&D) of buildings, use of clean of nest material, frequent egg collection, and egg washing, primarily with quaternary ammonium and/or chlorine compounds. Environmental monitoring for salmonella after C&D assures adequacy. Formaldehyde is not in use anymore because of regulatory restrictions.

Vaccines are routinely used prior to egg production to protect against respiratory diseases such as fowl cholera, Newcastle disease and infections by paramyxovirus 3. Avian influenza vaccine may be included in some areas. Biosecurity is the byword of a successful and disease-free breeder operation. Many programs have shower-in, shower-out facilities for employees and all personnel.

HATCHERY

Cleaning and disinfection are a high priorities and phenolic disinfectants are generally used. Phenolics are also used in foggers in work areas. Aspergillosis occurs in some areas of the country and is controlled with the Clinafarm^{R} smoke disinfection product when egg sanitation practices are not adequate. Antibiotic (gentamycin or spectinomycin) injection of poults is used to control egg transmitted bacterial infections and also helps to counter stress-related infections which occur in the young poult.

BROODING

Flock depopulation (all-in, all-out management) and complete C&D of the house are high priorities. A two-step process of washing first and then disinfecting is preferred in some operations. Concrete floors have increased in use because of greatly improved C&D characteristics. Phenolic and cresylic acid are the favored disinfectants for dirt (soil substrate) floors. Quaternary ammonium, organic iodine, and concentrated chlorine disinfectants are also used alone and in various combinations. Formaldehyde is not used anymore.

Ammonia is a primary cause of respiratory disease and also acts as a co-pathogen. Ammonia control has a high priority in colder climates where the ventilation rate is minimized to maintain room temperature. Control methods include removal of wet litter, mixing (rototilling) and rebedding of litter, and management of heat, ventilation, humidity, and water fountains. Sophisticated computer controlled systems are available and are utilized in some operations. Manual systems without fans may utilize a thermostatically controlled curtain minder.

Bordetellosis (turkey coryza, BART) is a significant problem in most areas depending on the environmental conditions. Vaccine is applied as a spray in the hatchery and in the drinking water in the brooder. Drinking water sanitation is a key factor in addition to all-in, all-out management and C&D. Continuous chlorination of drinking water is widely practiced. Shock chlorination and/or acid cleaning of the entire water delivery system between flocks is felt to be very important.

Colibacillosis may be triggered by a number of diseases. Newcastle disease (lentogenic virus) circulates in many turkey production areas and vaccines administered in the drinking water or by spray are used for protection. Hemorrhagic enteritis is caused by an immunosuppressing adenovirus which is routinely vaccinated against with the marble spleen virus of pheasants. Coccidiosis is controlled through vaccination (controlled exposure) or by coccidiostats in the feed or water.

GROWING AND FINISHING

Growing and finishing buildings are completely cleaned and disinfected annually or less often. Incomplete cleaning between flocks consists of physical cleaning using mechanical blowers followed by washing and disinfection. Treatment of the superficial litter may involve either partial removal (skimming) or mixing (rototilling) with the addition of new litter. Types of litter currently in use are wood shavings, and the hulls of rice, sunflowers or peanuts. A high concentration of round worm eggs or a heavy infestation of meal worms in built-up litter can result in a severe stress on a naive flock. This is corrected by depopulation (all-in, all-out management), and thorough C&D.

Dust in confinement buildings causes foreign body pneumonia, airsacculitis and aspergillosis. The dust is primarily feces which has dried to a light weight powder, along with some feather and skin dander and feed particles. After 10- to 12 weeks of age, turkeys in cool climate areas during the winter experience a dry environment with high dust levels. In warm climate areas during the summer heat, turkeys may experience excessive intake of dust from hyperventilation. Both conditions lead to respiratory disease ("gaspers") leading to mortality or airsacculitis condemnation at processing. In addition to hot weather cooling efforts, fogging or misting of the air and sprinkling of litter are used to control dust. It may also reduce breast blister formation. Growers in cool climates have found that lowering the building temperature, particularly for toms 10- to 12 weeks of age, reduces dust levels because of an increase in relative humidity. This results in healthier birds, less mortality, faster weight gain, and improved feed conversion and costs.

BIOSECURITY

Improvements in personnel and vehicular traffic control as well as sanitation practices have become standard in the industry. Cleaning and disinfection stations at the farm entrance and at each building are common even in areas with freezing weather and are major factors in the control of all transmissible diseases. Boots and coverall clothing, including hair nets, are in

use in most areas. Domestic and feral animals, particularly wild birds and rodents, are a constant control challenge. Sanitary disposal of dead birds has improved now in many areas with well-managed composting done on the farm.

PROCESSING

Using the monthly report of the USDA poultry processing condemnations, a state-by-state comparison reveals a significant variation in the airsacculitis condemnation category. Utilization of airsacculitis salvaging procedures affects the incidence of condemnation as well as the bottom line.

VENTILATION

Natural ventilation is the most widely used system. Curtain openings on both side walls in and east-west oriented building catch the southerly winds broadside in the summer time. Other openings for wind and convection of air include screened end wall and side wall doors and vents, as well as attic and ridge openings. Control of the openings may be either manual or automated with thermostats, stage controller and/or computer. Slow-speed, air-stirring fans may be mounted on the ceiling, side walls, posts or the center of the house and used with either manual or thermostat and timer controls.

Negative pressure ventilation using exhaust fans controlled by thermostats and/or timers is used in most areas of the country especially for brooding. System control varies from manual operation to automation with thermostats, timers, stage controls and sophisticated computer applications. Slow-speed, air-stirring fans and side wall curtains may also be used. Tunnel ventilation is an extreme application of negative pressure and is used along with fogging in some hot climate areas.

Positive pressure air exchange using "make up" air equipment has lost popularity due to the high costs of operation and maintenance.

"Positive air" ventilation which emphasizes air quality and not necessarily positive pressure air exchange has increased in popularity. It is a computer controlled, efficient pulsing system utilizing a "make up" air machine, center suspended horizontal circulation fans, automatic side wall curtains and end wall curtains for exhaust.

ACKNOWLEDGMENTS

I would like to recognize and thank the practicing industry veterinarians who responded to my inquiry as to their experiences and management practices, and whose information I have included in this paper.

Symposium Evaluation

Please take a few minutes to complete this survey and either leave the form on the table in the back of the presentation room, or mail the form to the address below. Your responses will have an impact on future symposia.

Scoring: 1 = strong yes, 2 = yes, 3 = neutral, 4 = no, 5 = strong no

Scoring	Question
1 2 3 4 5	The symposium on Respiratory Diseases of Chickens and Turkeys provided useful information.
1 2 3 4 5	The format of providing a mixture of basic and applied information was an asset.
1 2 3 4 5	The symposium had too much basic information.
1 2 3 4 5	The symposium had too much applied information.
1 2 3 4 5	The symposium was too long.
1 2 3 4 5	The depth of the information was sufficient for each topic area.
1 2 3 4 5	The diverse backgrounds of the speakers (industry, academia, government, and diagnostic laboratories) were an asset.
1 2 3 4 5	Including an international speaker brought the symposium a global perspective on poultry disease.

Additional comments:

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