POULTRY MEDICINE
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SEROLOGICAL EVIDENCE OF AVIAN METAPNEUMOVIRUS INFECTION IN COMMERCIAL BROILERS FROM THREE REGIONS OF MYANMAR

Y. H. Aung¹, N. Htun², H. H. Myint¹

¹ Department of Medicine, University of Veterinary Science, Yezin, Nay Pyi Taw, Myanmar
² Township Livestock Breeding and Veterinary Department, Athok Township, Livestock Breeding and Veterinary Department, Ministry of Livestock and Fisheries, Myanmar

Topic: 11. Poultry Medicine / Respiratory Diseases

Swollen head syndrome associated with avian Metapneumovirus (AMPV) in broilers and broiler breeders has been reported worldwide. However, to our knowledge, there is no report on AMPV infection in broilers in Myanmar. Since head swelling is frequently encountered in commercial broilers in Myanmar, the objective of the present study is to investigate whether AMPV is associated in respiratory infections of commercial broilers in Myanmar. Serum samples of broilers from 42 commercial broiler farms, 14 farms from each region, were collected from Yangon, Mandalay and Bago regions. Specific antibodies to AMPV were detected by commercial ELISA (BioChek, the Netherlands) and the data obtained were analyzed by Chi-square test. Four of 14, 11 of 14, and 8 of 14 farms were seropositive to AMPV in Yangon, Mandalay, and Bago regions, respectively. Seropositive to AMPV in Mandalay region was significantly higher \( (p<0.05) \) than that of Yangon region. The numbers of seropositive farms of 5-6 and 7-8-week-old broilers were significantly higher \( (p<0.05) \) than that of 3-4-week-old broilers. The number of seropositive farms of crossbred broiler strains were significantly higher \( (p<0.05) \) than that of purebred broiler strains. No significant differences \( (p>0.05) \) in numbers of seropositive farms among different flock sizes were observed. Seropositive to AMPV was observed in broilers with or without head swelling. Overall, it is the first study, which indicates serological evidence of AMPV infection in commercial broilers in Myanmar, since there have no history of anti-AMPV vaccination in commercial broilers in Myanmar.
AVIAN MYCOPLASMA VACCINATION - CURRENT PRACTISES AND BENEFITS INCLUDING DECREASED ANTIBIOTIC DEPENDENCE

C. Morrow

Technical, Bioproperties, Ringwood, Australia

Topic: 11. Poultry Medicine / Respiratory Diseases

Live mycoplasma vaccines are now the state of the art for mycoplasma control where mycoplasma freedom is impractical or uneconomic. In breeders the ultimate measure of successful control is if the progeny do not need to have antimycoplasmal antibiotics during production. In layers live MG and MS vaccines are the first step to allow the rearing and production periods to be antibiotic free. Prevention of glass top eggs (associated with a particular MS strain) in layers has been achieved with MS-H vaccine solving a specific problem. These benefits can be obtained without supplementary killed vaccines. On multiage production sites these vaccines can displace field strains and they can be looked at as increasing the resistance of the birds to wild strain infection. Horizontal transmission is negligible. Key features are

1. Vaccines need to be given by eye drop at least three weeks before expected challenge. The cold chain for delivery and storage needs to be respected.

2. Antimycoplasmal antibiotics can be expected to disrupt or decrease the immunity generated so they should not be used two weeks before vaccination or for the month after vaccination. Use after this period should be only on an as needed basis with pulse administration if possible.

3. MS can mimic anything MG can (use PCR for diagnosis) and needs to be controlled. Serology is often equivocal.

Improvements in production parameters including egg production, FCR of egg production, egg quality have been seen in trials around the world even in farms where infections were subclinical.
DETECTION AND MOLECULAR CHARACTERIZATION OF INFECTIOUS LARYNGOTRACHEITIS VIRUS IN LAYING HENS IN LIBYA

A. Asheg, A. Kammon, O. Trahuni

Poultry and Fish disease, Faculty of Veterinary Medicine-Tripoli University, Tripoli, Libyan Arab Jamahiriya

**Topic:** 11. Poultry Medicine / Respiratory Diseases

Avian Infectious Laryngotracheitis, caused by Infectious Laryngotracheitis Virus (ILTV), has been reported in laying flocks from Libya. More recently, outbreaks have occurred in Tripoli's. This study reports the application of PCR and DNA sequencing targeted to the p32 gene of ILTV using isolated virus via egg incubation. Samples were positive by PCR. DNA sequencing of samples evidenced homology of the amplified fragments with the p32 gene of ILTV. The results definitely confirmed the presence of ILTV in the birds during the outbreak. Further studies are needed to establish the sources of infection and to determine whether the detected virus was originated from vaccine or field virus strains.

Keywords: Avian infectious laryngotracheitis, Libya, p32, PCR, sequencing.
Composition of gut microbiota is known to influence many gut functions. However, most of the studies on gut microbiota in chickens were performed in broilers up to the age of 8 weeks and how dynamic or stabil is the gut microbiota composition in egg layers is nearly unknown. In this study we were therefore interested in the development of caecal microbiota in egg laying hens over their whole life, from a day of hatch till age of 60 weeks. Using the pyrosequencing of V3/V4 variable regions of 16S rRNA genes for microbiota characterisation, we were able to define 4 different stages of caecal microbiota development. The first one lasted for the first week of life and was characteristic by a high prevalence of Enterobacteriaceae (phylum Proteobacteria). The second stage lasted from week 2 to week 4 and was characteristic by nearly an absolute dominance of Lachnospiraceae and Ruminococcaceae (phylum Firmicutes). The third stage lasted from month 2 to month 4 and was characteristic by the succession of Firmicutes on the expense of Bacteroidetes. The fourth stage was typical for adult hens in full egg production aged 5 months and more and was characteristic by a constant ratio of Bacteroidetes and Firmicutes formed by equal numbers of the representatives of both phyla.
LOCAL ANTIBODY RESPONSES IN THE RESPIRATORY TRACT AND INTESTINE OF CHICKS VACCINATED WITH VARIANT IBV VACCINE CR88

R Elzlitni, G. Elhafi, R. Jones

Preventive Medicine, Tripoli University, Tripol, Libyan Arab Jamahiriya

Topic: 11. Poultry Medicine / Respiratory Diseases

Some important variant strains of IBV replicate in the intestine. However, commercial vaccines against these strains have never been examined for production of enteric immunity. Local virus-specific IgA, IgM and IgG were detected in serum, tears, and tracheal and intestinal washes from chicks vaccinated orally (simulating drinking water application) or by spray with CR88 live vaccine, using specific monoclonal antibodies. All were detected in these secretions and oral application was consistently more successful than spray. Virus-neutralising activity was found in all secretions including pooled secretions from the intestinal regions, suggesting an effective immune response. Results need to be compared with field trials with such vaccines.
The objective of this study was to investigate specific antibody responses to three commercial strains of infectious bursal disease virus (IBDV) vaccine (B38, MB-Vac and D78) in two commercial strains of broilers (Lohmann meat and 120 Ross308). One-day-old 120 Lohmann meat and 120 Ross308 broilers were randomly divided into 4 groups in each strains: Loh A and Ross A, Loh B and Ross B, Loh C and; Ross C, and Loh D and Ross D. Broilers from the groups, Loh A and Ross A, were kept as unvaccinated control. At 21 day of age, broilers from the Loh B and Ross B, Loh C and Ross C, and Loh D and Ross D groups were vaccinated with B38, MB-Vac, and D78 strains of IBD vaccines, respectively, by oral drop. Six serum samples from each group were collected at 7, 14, 21, 28, 35, 42, and 49-day of age. Specific antibody response to IBDV was monitored by commercial ELISA. Specific ELISA antibody response to IBDV in Lohmann strain was significantly higher ($p<0.05$) than that of Ross308 strain at 49 day of age. Specific antibody response to MB-Vac was significantly higher ($p<0.05$) than those of B38 and D78 at 35-day of age, whereas antibody response to D78 was significantly higher ($p<0.05$) than those of B38 and MB-Vac at 42-day of age. Overall, both strains of broilers response well to the IBDV vaccines and all three strains of IBDV vaccines induce specific antibody response in commercial broilers.
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QUANTITATIVE VERSUS QUALITATIVE DETECTION: PROS AND CONS IN VIEW OF A MULTIPLEX QUANTITATIVE REAL-TIME PCR ASSAY

I. Davidson, A. Al-Touri, I. Reibshtein

Division Of Avian Diseases, Kimron Veterinary Institute, Bet Dagan, Israel

Topic: 11. Poultry Medicine / New Challenges and Technologies in Diagnostics

Chicken anemia (CAV) and Marek’s disease (MDV) are widespread, ubiquitous and economically significant as single- or dual-virus infections. They often accompany various pathogens and affect health directly, causing tumors, anemia, delayed growth, and indirectly, by immunosuppression and aggravating other diseases.

We developed a real-time multiplex PCR (RT-PCR) for their simultaneous quantitative detection. The assay applicability and diagnostic value was assessed using organs of experimentally infected and commercial chickens. Both qualitative cPCR and quantitative real-time PCR were applied on same samples. The RT-PCRs for MDV and for CAV alone, or in multiplex, were more sensitive then the respective cPCRs.

While in the experimental infection model the qualitative positive and negative samples contained distinctive viral copy number, and did not overlap, samples derived from commercial flocks had a broad "grey area". The wide-ranging clinical situations and infection statuses, as well as the infection timing relative to sampling, might account for the virus presence in low viral copies.

In conclusion, while the cPCR was unambiguous in evident clinical cases, the RRT-PCR detected low to medium viral copy numbers also in chickens without evident clinical signs. Due to the MDV and CAV importance in inducing sub-clinical immunosuppression, the virus quantitative estimation is valuable in clinical and in sub-clinical infections of commercial poultry.

The ability to quantitate avian pathogens raises dispute regarding diagnostic significances in face of various clinical manifestations, and various veterinary policies.

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MICROWAVE OR AUTOCLAVE TREATMENTS DESTROY THE INFECTIVITY OF INFECTIOUS BRONCHITIS VIRUS AND AVIAN PNEUMOVIRUS BUT ALLOW DETECTION BY RT PCR

G. Elhafi¹, C. J. Naylor², R. C. Jon³, C. Savage⁴, R. Abdalla Elzlitne⁵

¹Department of Veterinary Pathology, University of Liverpool, Leahurst, Neston, South Wirral, CH64 7TE, UK,
²Tripoli University Tripoli-Libya

A method is described for enabling safe transit of denatured virus samples for polymerase chain reaction (PCR) identification without the risk of unwanted viable viruses. Cotton swabs dipped in avian infectious bronchitis virus (IBV) or avian pneumovirus (APV) were allowed to dry. Newcastle disease virus and avian influenza viruses were used as controls. Autoclaving and microwave treatment for as little as 20 sec destroyed the infectivity of all four viruses. However, both IBV and APV could be detected by reverse transcriptase (RT)-PCR after autoclaving and as long as 5 min microwave treatment (Newcastle disease virus and avian influenza viruses were not tested). Double microwave treatment of IBV and APV with an interval of 2 to 7 days between was tested. After the second treatment, RT-PCR products were readily detected in all samples. Swabs from the tracheas and cloacas of chicks infected with IBV shown to contain infectious virus were microwaved. Swabs from both sources were positive by RT-PCR. Microwave treatment appears to be a satisfactory method of inactivating virus while preserving nucleic acid for PCR identification.
SURVEY ON THE AVIAN BREEDING AND PATHOLOGY IN THE EAST OF ALGERIA

N. Adili\textsuperscript{1}, M. Melizi\textsuperscript{1}, O. Bennoune\textsuperscript{1}, T. Khenenou\textsuperscript{2}, K. Hadji\textsuperscript{3}, W. Touirat\textsuperscript{3}

\textsuperscript{1} Laboratory ESPA, Veterinary Medicine Department, El-Hadj Lakhdar University, Batna, Algeria
\textsuperscript{2} Veterinary Medicine, University of Souk Ahras, Souk Ahras, Algeria
\textsuperscript{3} Veterinary Medicine, El-Hadj Lakhdar University, Batna, Algeria

**Topic:** 11. Poultry Medicine / Hygiene and Biosecurity

**Aim:** This survey has been conducted in order to know the sanitary situation of avian breeding in the East of Algeria, the main avian diseases, the failing of vaccination protocols and the difficulties in the establishment of accurate diagnosis of these pathologies.

**Materials and methods:** This survey has been made up in the form of a questionnaire sent to veterinary surgeons in the East of Algeria; in order to collect information on the diagnostic methods, the management and the vaccination protocols. Furthermore, this survey is devoted to study the frequency on the most important avian diseases and the failing of vaccination in the prevention of these diseases.

**Results:** A meticulous analysis of the survey results showed the frequency of several pathologies of bacterial, viral and parasitic origins. The failing of vaccination protocols have also been observed in this region in particular vaccination against infectious bursitis. Indeed, this survey has revealed that the veterinarians do not apply the manufacturer’s recommendations concerning the use of vaccines. Moreover, the diagnostic methods are limited to the clinical signs and necropsy findings. Therefore, the diagnosis is based on the success or the failing of the therapeutic methods.

**Conclusion:** This strategic sector of agricultural activity suffers from many limiting factors and requires a profound popularisation of the avian breeders in order to familiarise them with the modern management methods. Furthermore, new diagnostic methods should be learnt by the local veterinarians in order to deal with efficacy with main avian pathologies of the region.
NATURALLY OCCURRING MYELOID LEUKOSIS IN COMMERCIAL BROILER CHICKENS

M. P. Franciosini, P. Casagrande Proietti, P. Coliolo, F. Veronesi, L. Leonardi, M. L. Marenzoni

Veterinary Science, Faculty of Veterinary Medicine, Perugia, Italy

**Topic:** 11. Poultry Medicine / Respiratory Diseases

Avian Leukosis-Sarcoma (ALS) Complex includes a variety of transmissible neoplasms of birds, caused by *Retroviridae* family. Lymphoid leukemia is the most common form of the disease, but in recent years myelocytomatosis and myeloblastosis have been increasingly reported in heavy breeders, in commercial hens, and more rarely, in broiler chickens. AL virus subgroup J is considered the main responsible for myeloid leukemia and myelocytomatosis. This paper reports on myeloid leukemia cases, accidentally observed on two consecutive productive cycles in broilers aged between 40-50 days and coming from the same breeders. The histological study revealed pleomorphic cells with eosinophilic granulations irregularly distributed in liver parenchyma. Ultramicroscopic studies from paraffin embedded sections confirmed the histological results, showing cells with cytoplasm filled with several electron-dense and homogenous granules, indicative of myeloid lineage. Viral particles, 60-70 nm in diameter, detected in the nucleus, were suggestive of avian adenovirus while no viral particles related to retroviruses were found. This result is not surprising since the mieloid leukemia virus is not usually found in substantial amount and in our case there was not availability of fresh material for a more detailed ultrastructural study. The presence of adenovirus with immunosuppressive action could play a role in the early occurrence of myeloid leukemia. The occurrence of these tumors in chickens from the same breeding supports the hypothesis of vertical transmission, typical of Retrovirus infections. Further studies are required to establish the actual role of Adenovirus in determining the neoplastic disease.
STUDY OF THE MINERAL COMPOSITION OF THE SHELL EMBRYONIC EGG

B. Bani, M. Mohamed

University of Batna, Batna, Algeria

Topic: 11. Poultry Medicine / Food Safety

Considering calcium is not the only mineral exhausted by the embryo during the embryonic development, we propose to evaluate the rate and the behavior of minerals that constitutes the shell of embryonic egg.

For that; a study of the mineral composition of the shell of embryonic egg is carried out according to the embryonic development stages, then compared with that of egg for consumption.

Incineration and acid-attack procedure were performed on the embryonic-egg shells at the stages: 1, 7, 14, and 21 days of incubation, to determine calcium, magnesium, manganese, zinc and copper content by atomic absorption spectrophotometry, whereas the phosphorus content was determined by visible spectrophotometry.

Our results are largely lower than those reported in the bibliography with regard to the average content of the embryonic-egg shells of manganese magnesium and calcium. While, the rate of copper and zinc increased from 1st to the 21st day of incubation; phosphorus presented a behavior independent of other minerals with a stable rate throughout the incubation period.
AMELIORATIVE EFFECTS OF L-CARNITINE AND VITAMIN E UPON TOXICOLOGICAL ALTERATIONS INDUCED BY OCHRATOXIN A (OTA) IN WHITE LEGHORN COCKERELS.

Z. Abidin¹, M. Z. Khan², A. Khatoon³, M. K. Saleemi², A. Khan², I. Javed¹

¹ Central Reference Lab, Veterinary Research Institute, Lahore Cantt, Pakistan
² Department of Pathology, Faculty of Veterinary Sciences, University of Agriculture, Faisalabad, Pakistan
³ Department of Physiology and Pharmacology, Faculty of Veterinary Sciences, University of Agriculture, Faisalabad, Pakistan

**Topic:** 11. Poultry Medicine / Food Safety

A total of 240 cockerels were selected and divided to 12 groups containing 20 birds each. Each group was treated with different levels of OTA, L-carnitine or vitamin E or their combinations. Birds treated with OTA were depressed and less attractive to feed having ruffled feathers. Body weights and organ weights of the groups treated with OTA were also depressed. Hematology of toxin groups showed a decrease in PCV, Hb, TEC and TLC moving the birds in an anaemic state. Total proteins and albumen concentrations in the serum of toxin groups were significantly lower while serum urea and creatinine in toxin groups was significantly higher than control. Liver of control group showed normal hepatocytes and normal sinusoidal spaces. Nuclei were normal with exception of only few pyknotic nuclei. In kidneys, tubular epithelial cells had normal nuclei and urinary spaces were clear and dilated. In OTA treated groups, the sinusoidal spaces were congested and hepatocytes were pyknotic while in kidneys, there was pyknosis of nuclei of tubular epithelial cells and urinary spaces were also congested. All these alterations and lesions were more severe at higher doses (2.0 mg/kg OTA) while less severity was observed at low levels (1.0 mg/kg OTA). Results confirmed that L-carnitine and vitamin E given alone or combination with 1.0 mg/kg OTA ameliorated OTA induced alterations in behavioural parameters, body weight gain, and organ weight, feed intake, haematological, serum biochemical and histopathological parameters. This amelioration, however, was not seen at 2.0 mg/kg OTA suggesting a dose dependent protection.
INVESTIGATE THE POSSIBILITY OF USING PISTACHIO KHINJUK MEAL IN BROILERS DIET

S. Parsanasab¹, F. Zamani², J. Porreza³, M. Faghani¹

¹ Animal Science, Azad University Shahrekord Baranch, Shahrekord, Iran
² Animal Science, Agriculture and Natural Resources Research Center, Shahrekord, Iran

The study was conducted to determine the effects of dietary supplementation Pistacia khinjuk meal (PKM) on growth performance and carcass fatty acids profile of broiler chicks. One hundred twenty 1-d-old Ross-308 male chickens were weighed and in a completely randomized design divided into 4 groups each with 3 replicates of 10 birds. group (1) was fed basal diet without PKM, group(2) was fed on 5% PKM0 with basal diet, group (3) was fed on 10% PKM with basal diet and group (4) was fed on 15% PKM0 with basal diet. Feed intake, body weight changes, feed conversion ratio and daily gain were investigated. At the end of the experimental period all birds were killed and carcass trait was measured. Two carcasses were randomly selected from each replicate and Soft tissues were separated and well mixed with grinder to extract lipids and fatty acids measured by GC mass. The result shown feed intake and feed conversion ratio was significantly (P<0.05) lower in group 2 than other groups. Moreover final body weight and carcass weight of birds that fed diet containing 5% PKM was higher than control group(P<0.05). with increasing PKM in diet increased significantly oleic acid(C18:1) and linoleic acid(C18:2) in carcass fat. The results showed that the pistachio khinjuk meal can well be used in broiler diets.
AFLATOXICOSIS RETARDS TISSUE DEPLETION OF TILMICOSIN IN TURKEYS THROUGH IMPAIRED CLEARANCE FROM THE BODY

M. Ismail, A. Y. AL-Taher

Physiology, Biochemistry and Pharmacology, King Faisal University, Al-ahsa, Saudi Arabia

**Topic:** 11. Poultry Medicine / Food Safety

Aflatoxicosis are deleterious to poultry and their contaminant in feed is practically unavoidable. Among different poultry species, turkeys are shown to be the most susceptible to aflatoxin B1. This work has been done to elucidate the impact of aflatoxin B1 on pharmacokinetic and tissue depletion of tilmicosin in turkeys. Pharmacokinetic of tilmicosin and tissue depletion of residue following dosage of were studied in turkey fed aflatoxin free diet and aflatoxin contaminated diet at two contamination levels (300 and 600 ppb). Tilmicosin concentration in plasma and tissue were analysed by using HPLC method. Finding of the present study showed a significant increase in the absorption half-lives and elimination half-lives, plasma concentration, time to maximum concentration of tilmicosin in birds fed diets contaminated with low and high contaminant of aflatoxin. The withdrawal time of tilmicosin was calculated according to the European committee statistical approach with tolerance limit of 95%. The estimated withdrawal times for tilmicosin in different tissues studied (meat, fat, kidney and liver) were significantly higher in birds fed low contamination and high contamination levels of aflatoxins. Values of 12.7 days, 13.1 days, 13.7 days and 8 days for meat, fat, kidney and liver withdrawal times of tilmicosin in birds fed aflatoxin free diet were significantly increased to 18.6 days, 18.1 days, 19.8 days and 14 days in birds fed low aflatoxin contaminant level diet and 23.1 days, 21.5 days, 23.7 days and 25 days in birds fed high level of aflatoxin contaminant diet, respectively.
USE OF ANTIMICROBIALS IN POULTRY IN CZ - CURRENT STATUS AND PERSPECTIVE OF POSSIBLE APPROACHES FOR THE FUTURE

L. Pokludova¹, J. Bureš¹, A. Hera¹,²

¹ Department of Marketing Authorisation, Institute for State Control of Veterinary Biologicals and Medicaments, Brno, Czech Republic
² Department of Pharmacology and Pharmacy, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic


Intensive poultry farming, especially fattening broilers belongs among the groups, in which mass medication is used for treatment and prevention of infectious diseases, therefore the use of antimicrobials here is of great concern.

Antimicrobials from 10 pharmacological groups are authorised and used for the treatment and prevention of poultry diseases in poultry farming in the Czech Republic, i.e. practically whole portfolio of pharmacological groups is covered. It should be emphasized that any cephalosporins are neither authorized nor used in the poultry in the Czech Republic. As the maximum residue limits have not been established for all substances, list of actives used in laying hens is limited. Majority of antimicrobials is used via medication of drinking water.

Use of the antimicrobials differs across different types of farming - the highest use is in fattening broilers, less in laying hens and parent breeding flocks.

Main diagnosis in which antimicrobials are used are necrotic enteritis, colibacillosis, mycoplasmosis, less frequently other respiratory and gastrointestinal tract infections.

Based on the data on sales of antimicrobials (2011; kg of active substances) following antimicrobials are the most frequently used in poultry tetracyclines, amoxicillin, sulphonamides (usually in combination with trimethoprim), (fluoro)quinolones, macrolides, colistin and tiamuline.

It should be stressed that further measures, which will lead to the decrease of the need of use of antimicrobials and their more rational use should be promoted.

Positive examples both from other EU countries and from the Czech Republic show that production of poultry with minimal and responsible use of antimicrobials is possible.
INCREASING ANTIBIOTIC RESISTANT ESCHERICHIA COLI: MULTI-RESISTANT ESCHERICHIA COLI ISOLATED FROM CHICKENS AND PIGLETS FROM SMALL SCALE FARMS


University of Veterinary Science, Myanmar Veterinary Association, YEZIN, NAY PYI TAW, Myanmar

A total of 382 generic Escherichia coli isolates were recovered from chickens and piglets of small scaled farms at different locations in Myanmar. (182) E. coli isolated from chickens and (200) isolates from piglets. E. coli isolates from chickens and piglets were tested for antibiotic sensitivity to (12) antibiotics. Among them a total of 132 strains of E. coli isolates were studied for plasmid DNA analysis. 108 (81.8%) of E. coli isolates with plasmids and 24(18.2%) of E. coli isolates without plasmids were observed. 14 (10.5%) carried two plasmids and 19 (14.4%) contained as many as three plasmids. Escherichia coli isolated from chickens was investigated as resistance to trimethoprim (98.9%) while Escherichia coli isolates showed a high percentage of antimicrobial resistance to ampicillin (91% and 94.5%) and tetracycline (95% and 96.7%) in piglets and chickens, respectively. E. coli isolates appeared resistance to cepharadine (85% and 96.7%) while moderate resistance to chloramphenicol (64% and 74.4%) and kanamycin (55% and 68.6%) in piglets and chickens, respectively. Multiple Antibiotic Index (MAR) ranged 0.1~1.0. (MAR) E coli was observed as 141/382 (36.7%) and 96/382(25.1%) resistance to 12 and 11 antibiotics, respectively. The data indicated that environmental sources as an important factor for increased antimicrobial resistance even antimicrobials were used very rare or none in those farms. The study suggested that colonization by multiresistant E. coli in chickens and piglets may not only result from antibiotic supplements in feed but also from acquisition of these organisms and transfer of antibiotic resistance genes from environment.