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Full Length Research Paper

Pseudomonas infection in chickens

Fekadu Kebede

National Veterinary Institute, Debrezeit, Ethiopia. E-mail: mycogs1@yahoo.com.

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Pseudomonas aeuroginosa **is a motile, gram negative, oxidase positive bacteria, grows well on MacConkey agar media. It is a rod shaped bacteria occurring singly or in short chains, microscopically, margins of the colonies are having hook like projections. The organism is a strict aerobe, also grows readily on other common bacteriological media. The bacteria usually produces a water soluble green pigment composed of fluorescent and pyocyanin with a characteristics fruity odor. The organism is ubiquitous, often associated with soil, water and humid environment. In this case study, the organism was isolated from young chickens and dead embryos. The disease causes high mortality in newly hatched chickens and mass death of embryo at later stage. Our bacteriological results were consistence with the published literature. The experimental trial on different age groups has showed that the isolated causative agent (*P. aeuroginosa*) was found to be highly virulent to the young chickens (1 - 10 day old) and less pathogenic to chickens of 11 - 20 day old. The older chickens (>20 days) were found to be resistant to the infection. The agent more likely invades the host organism through wound and/or respiratory route. The organism was found to be resistant for the most common antibiotics, but it was found highly sensitive to Polymixin B and Amicacin which gave wide growth inhibition zone of 1.5 and 2 cm, respectively. Prevention of the *Pseudomonas* infection in the farm can be achieved by taking all the necessary bio-security measures.**

Key words: *Pseudomonas aeuroginosa*, embryos, hook like projection colonies

INTRODUCTION

Infection may gain entrance to a flock of birds from various sources, some of which may more conveniently enter because of errors in management and industry practices. The microenvironment of chickens' house is ideal for growth of various microorganisms and thus serves as a potential source of infection in poultry farms. Specially, those organisms which can survive for a longer time in the environment are to be considered as the main environment associated disease threat because of the difficulty in eradicating them from the chickens' house. Such organisms are resistant to various antibiotic treatment and conventional disinfectants.

Pseudomonas is the one which can be cited as a good example of environment associated infection and may cause a serious problem in poultry farms. *Pseudomonas aeruginosa* is a motile, gram-negative, non-spore forming rod occurring singly or in short chains under microscopic examination. The organism is strict aerobic that grows readily on common bacteriologic media; usually producing a water soluble green pigment composed of fluorescent and pyocyanin with a characteristic fruity odor. The organism is ubiquitous, often associated with soil, water, and humid environments. Generally, it is considered to be an opportunistic organism that produces respiratory infections, septicemia and other forms when introduced into tissues of susceptible birds.

P. aeruginosa is the most common pseudomonad causing infections. It can be highly virulent causing 50 - 100% mortality in experimentally inoculated 4-week-old chickens.

In this case study, the disease of *Pseudomonas* induces a significant economic loss to the farm by causing high mortality of newly hatched chickens and death of embryo at later stage.

METHODOLOGY

The case study was conducted on sick, dead chickens, egg embryos collected from one commercial farm in Ethiopia. The activities were: Case definition based on the clinical signs; Post-mortem examination

from fresh dead and sacrificed sick chickens; Isolation and characterization of the isolated organisms; Experimental trial on different age groups of chickens and embryonated eggs.

Clinical sign

A number of sick chickens from the farm at different stage of the disease were examined and all clinical signs associated with the disease were recorded accordingly.

Post-mortem examination

Fresh dead and scarified sick chickens were subjected for pathological examination.

Isolation of the organism

Specimen used: Blood from heart and liver. Growth media: Tryptose agar, MacConkey agar and Tryptose broth, Gram stain, Oxidase test, Motility test. Biochemical tests: O- F test, Cimon citrate, MR-VP, Indol, and urease. Antibiotic discs for sensitivity tests used were: Tetracycline, Clindamycin, Erythromycin, Polymyxin B, Vancomycin, Amicacin, Methiciline, Penicillin, Nalidixic acid, Cffoxicilin and Nitrofurazone

Bio- prob

Inoculation of the isolated organism into mice (2), day old chickens (2), and adult hen (2) using intra-peritoneum route, 0.2, 0.2, 0.4 and 0.5 ml of the culture suspension, respectively, and two control animals were assigned from each spp/group

Experimental trial

The trail was carried out on different age groups of chickens for virulence test of the isolated organism. The chickens were randomly distributed in 3 groups constituting 10 chickens /group including controls in each group.

Embryonated egg inoculation with bacterial suspension, (0.2ml)

Early stage (10 days) - 5 eggs (2 control)
Mid stage (15 days) - 5 eggs (2 control)
Late stage (19 days) - 5 eggs (2 control)

RESULT

The results comprised of clinical case definition, post-mortem findings, bacteriological analysis, and experimental findings.

Case definition

The prominent feature of the disease in affected farm has been associated with high mortality, particularly in newly hatched chickens (1 - 10 day old) and death at later stage of the embryo. Depression, loss of appetite, rough coat,stands with closed eyes and death after few hours in a day were also observed in most infected chickens by the disease. The death in day old chickens were found to be much faster than chickens in 5-10 days old. Chickens above one month age were found to be resistant to the disease.

Post-mortem findings

Gross pathological lesions in chickens result in death due to *Pseudomonas* infection, were abdominal distention and the cloacal region was tensed and bulged. There were excess accumulations of green gelatinous fluid covered with thin membrane in the abdominal cavity. In most cases, the liver has pale color and more expressively it has a para-boiled appearance.

Bacteriological analysis

Blood and liver samples were inoculated in tryptose agar and incubated at 37^{O}C for 24 h. The organism was found to grow very well within 8 h.

Colony morphology

The color of the media was observed to have change into light green. It also assumes a medium size with irregular margin and when kept for a long time, appears light brown in color. Gives sweet fruity odor which is a specific characteristic of the organism

The colonies have hook like projection at the periphery/ margin and when it was examined under low magnification power, the center appears to be more dense. Specially hook like projections are found to be a unique colony characteristic of the organism (*P. aeuroginosa*).

Bio-prob

The virulence of the isolated organism was tested in laboratory animals including mice, rabbit and hen. Of the inoculated mice one was found dead after 12 h and the other one was found to be very sick. The inoculated organism was re-isolated from the animals.

All the inoculated day old chickens were found dead after 10 - 12 h. The control group from both species remained healthy. The rabbit and adult hens were not seriously sick except slight depression which was observed for a short time.

Eggs inoculation

The experiment was conducted on embryonated egg

Table 1. Experimental group and route of infection.

Group	Total no. of chickens	(Sub-group) # of chickens/route/dose				Age (day old)
		(1)	(2)	(3)	(4)	
1	10	3/Sc/0.3	3/per-os/0.4	2/intra-peritonium/0.2	2/control	1-10
2	10	3/Sc/0.4	3/per-os/0.5	2/intra-peritonium/0.3	2/control	11-20
3	10	3/Sc/0.5	3/per-os/0.6	2/intra-peritonium/0.4	2/control	21-30
Total	30	9	9	6	6	

Table 2. Primary characterizing test.

Type of tests	Result
Gram's reaction	Gram negative
Oxidase	Highly positive
Motility	Motile
Growth on MacConkey	Non-lactose fermenter

Table 3. Biochemical tests.

Time (h)	O-F test	Citrate	H_2S	Indol	MR	VP	Urease
24	Oxidative	+ve	-Ve	-Ve	-Ve	-Ve	-Ve
48		+ve	-Ve	-Ve	-Ve	-Ve	-Ve

Table 4. Virulence test.

Route of infection	# of chickens/ group	Age group					
		1-10 days		11-20 days		21-30 days	
		Sick	Dead	Sick	Dead	Sick	Dead
Sub-cutaneous	3	All	All (12 h)	All	Two (12 h)	No	No
Intra-peritoneal	3	All	Two (12 h and one after 24 h)	All	One (12 h)	No	No
Per-os	3	All	Two (48 h)	No	No	No	No
Control	3	No	No	No	No	No	No

In most bacteriological and poultry disease text books, *P. aeruginosa* is not included or has given little emphasis associated with poultry diseases. However, in our findings, *Pseudomonas* infection was found to be a serious problem because it caused high mortality in newly hatched chickens and death of embryos at later stage.

Generally, *Pseudomona*s is considered to be an opportunistic organism that produces respiratory infection, sinusitis, eratitis/keratoconjuctivitis and septicemia and it becomes an infection when it is introduced into tissues of susceptible hosts (Diseases of Poultry, 2003).

In our findings, *P. aeruginosa* caused high mortality in day old chickens and death of the embryo in shell.

The bacteriological results are fully consistent with text published results. Hook like projections at the periphery of the colonies is a unique characteristic, this may be taken as the first explanation of colonies morphology of the organism. Literature evidence showed that, young birds are more susceptible, as are severely stressed or immuno-deficient birds. Concurrent infections with viruses and other bacteria, especially mycoplasmas are common and may enhance the infection of *Pseudomonas.* Morbidity and mortality rate are usually 2 - 10% but can be much higher (100%).

In our experimental findings, chickens in the age of 1 - 10 day were found to be highly susceptible to the *Pseudomonas*, and less susceptibility was observed in chickens in the age range of 11 - 20 day old. Chickens in age range of 21 - 30 day old were found to be resistant to the organism by all the routes of infection. The study

proved that infection through ingestion is not found to be a main route of infection. All sick and dead chickens were infected by sub-cutaneous and intra-peritoneal route. Most probably, the infection of *Pseudomonas* occurs through wound or umbilical cord during hatchery time from the environment or it can penetrate the egg shell at later stage of embryo and cause death.

The isolated *P. aeuroginosa* was found to be resistant to various antibiotics except Polymyxin B and Amicacin. It is also known as antibiotic resistant agent.

CONCLUSION AND RECOMMENDATIONS

Pseudomonas infection is not included as a serious infection of chicken in most poultry text books, but as per our study the disease can cause a serious economic problem in the poultry farms. We observed high mortality in newly hatched chickens and death at later stage of embryos in poultry farms. These findings may be the first report in the country associated with *Pseudomonas* disease in poultry farms.

P. aeuroginosa is considered to be an environmental infection and it is found in soil, water, feed and farm equipments. It is however difficult to clear the farm from the organism since it has high resistant to various antibiotics and may be resistant to conventional disinfectants. Hence, prevention of *Pseudomonas* invasion is an indispensable duty to any farm. The farm management should take stringent measures against all the possible sources of infection. The measures may include, the farm workers should be trained on how to avoid environmental associated infectious diseases and use of disinfectants periodically in hatcheries, incubators and house environment. Since, the poultry farm is ideal for the growth of micro-organisms, there should be close inspection for pathogenic organisms. Generally, the farm management should be committed in implementing all the necessary bio-security measures.

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