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The efficacy of the autogenous fowl cholera killed aluminum hydroxide vaccine in ducks in Iran

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SUMMARY

A fowl cholera killed aluminum hydroxide vaccine was Prepared with a local strain. Vaccinated birds were challenged, by contact exposure method, with **Pasteurella multocida** virulent strain type A_1, which had been isolated from a fowl cholera outbreak in the northern Part or Iran.

The vaccinated ducks showed a high level of immunity against the challenge up to the fifth month post-vaccination.

INTRODUCTION

fowl cholera is one of the earliest reported diseases of fowls. Acquired immunity from a killed vaccine has been a subject of much interest to many workers.

Heddleston and Resinger (1) showed that adsorbed monovalent vaccine stimulated and maintained a high level of immunity for one year. In other experiments they reported that aluminum hydroxide vaccine was of questionable immunizing value.

A brief review of the literature indicates that the method of challenge used most widely in both turkeys and chickens is swabbing of the nasal cleft with a cotton swab dipped with the challenge inoculum (1, 2, 3, 4). Alls, Appleton and Ipson reported a bird contact method of challenging turkeys with **P. multocida** (5).

In the present study a killed vaccine was applied to the ducks by inoculation. Vaccinated ducks were then exposed to virulent strain of **Pasteurella multocida.**

MATERIALS & METHODS

Cultures:

The strain of **Pasteurella multocida** used for bacterin production and for challenge had been recently isolated from an acute fowl cholera outbreak in

ducks, geese, chickens and turkeys in northern part of Iran (6). The isolated strain was identified biochemically and serologically as **P. Multocida** type A_1*. The strain was pathogenic for mice, rabbits, ducks and chickens. The strain was held in a lyophilized state until further use.

Vaccine preparation:

Freeze dried strain of **P. multocida** (strain type A_1) was reconstituated in nutrient broth and streaked on nutrient agar plates. After incubation, for 24 hours at 37° C, a single smooth iridescent colony was transferred to a nutrient broth and after 24 hours incubation, it was subcultured on yeast agar medium flasks and incubated for twenty four hours. Pure Roux flasks cultures were harvested in distilled water. The glass beads were used to remove the cells from the surface of the agar. Densities of bacterial suspension were identified by spectrophotometer equipment and colony count method. Formalin was added to the harvested suspension to make a concentration of 0. 25% and they were kept at + 4° C until use.

Bactrial suspension was adsorbed onto 10% aluminum hydroxide and the final product contained at least 10000 million organisms, as recommended by Heddleston, as a vaccinal dose (3). Sterility and safety tests were carried out by culture and inoculation into rabbits.

Vaccination:

Two flocks of twenty seven native ducks and fifty white pekin ducks were vaccinated by aluminum hydroxide fowl cholera vaccine into breast muscle with one vaccinal dose. Vaccinated ducks were divided into six groups for chalenge tests.

The challenge cultures were prepared forty eight hours before the day of challenge. Freeze dried challenge strain was resuspended in nutrient broth and streaked for colony isolation on nutrient agar and blood agar plates. After twenty four hours incubation at 37°C the typical **Pasteurella** colonies with green iridescence were transferred with a wire loop to five ml of nutrient broth and incubated for 24 h. The challenge culture (type A1) densitiy was found to be 1. 5 – 2×10^9/ml organisms by colony count method and has been used to infect the seeder birds.

Vaccinated ducks were challenged 21 days after vaccination. One or more of the unvaccinated controls in each group of ducks were used as the seeder birds (5). Inoculation of seeder bird was carried out with cotton swab which was dipped into the inoculum and then rubbed into the palatine cleft. Inoculated seeder birds were released to mingle with the vaccinated ones and the unvaccinated controls. The seeder brids died during 48 h. post challenge

* Isolated strain was identified as type A^1 in Veterinary Research Institute in Malasia

and were left for forty eight hours in the challenge room. All of the vaccinated and unvaccinated ducks were checked for two weeks post challenge.

Ducks:

The total ducks used in the experiments were forty four native and seventy three white pekin ducks which were obtained from Heidar-abad animal husbandry research Institute and a private duck farm. These birds were approximately three months old and had not previously been vaccinated and the flocks had no detectable antibody to **P. multocida.** Ducks were selected and marked at random with no regard to sex. Unvaccinated control birds were marked and kept in the same unit as the vaccinated ones, They were housed in a concrete-building room and each group of the birds was challenged in an individual room of 6×5 ft dimensions. Ventilation and other conditions were fairly good. Table 1 shows the results of the experiments.

Table 1. Results of contact exposure challenge of vaccinated ducks with **Pasteurella multocida** Type A_1 with palatine-cleft swab infection of the seeder birds.

breed	interval between vaccination and challenge	S/V	M/C	M/S	Percentage of the immune
1 native	21 days	6/7*	3/3	1/1	85.5
2 native	21 days	6/7	3/3	1/1	85.5
3 pekin	2 months	10/15	4/6	2/2°	67
4 native	3 months	11/13	6/6	2/2	84.6
5 pekin	4.5 months	11/15	4/6	4/4	73.5
6 pekin	7.5 months	8/20	2/3	2/2	40

S/V = number of survived per number of challenged in vaccinated birds.
M/C = number of mortality per number of challenged in controls.
M/S = number of mortality per number of challenged in seeder birds.
* = one died with nervous symptoms.
° = one died on the fifth day post challenge.

Result

As Table 1 shows, 85.5 percent of the vaccinated ducks in group 1 and 2 resisted the contact exposure challenge. On the ninth day only one duck of group 1 was showing signes of nervous symptoms like terticolis and torsion of the head and death occurred on the thirteenth day. No **pasteurella** was isolated and no lesions were observed in the microscopic examination of the brain, therefore, it was not included in the analysis.

In trial 3, one of the seeder birds died on the fifth day post challenge and pure **P. multocide** was isolated from the heart blood. In all of the experiments dead animals were autopsied and positive cultures of **Pasteurella** were obtained from their livers and heart blood.

The reason for the proportion of survivors in control groups of pekin ducks in the post-vaccination challenge may have been due to some individual resistance and enviromental conditions.

DISCUSSION

The immunity produced by a killed aluminum hydroxide vaccine was evaluated in native and white pekin ducks under a research project.

A vaccinal dose conferred a high degree of immunity in vaccinated ducks. The immunity level gradually decreased after the fifth month post vaccination.

Similar results of the immunity response were obtained from some infected flocks which experimentally were vaccinated by the aluminum hydroxide vaccine in north of Iran (6).

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