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Epidemiological and Immunological Studies on *Pasteurella multocida* in Rabbits at Upper Egypt

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SUMMARY

Pasteurella multocida is one of the most serious pathogens of rabbits; it causes considerable economic losses in rabbits production units. So this study was planned to spotlight on isolation, identification of *Pasteurella multocida*, serological diagnosis and confirmation of *Pasteurella multocida* by using PCR and how to control the disease.

To establish this goal a total of 120 samples of blood and nasal swabs (60 samples from apparently healthy rabbits and 60 samples from clinically diseased dead or scarified ones) were collected from private farms at different localities in Sohage governorate.

All samples were submitted for bacteriological examination, by conventional culture method. Recovery of Pasteurella isolates from apparently healthy rabbits and diseased ones were 6 (10%), 9 (15%) respectively, with total incidence of 15 positive cases (12.5%).

Also the isolated organisms were identified on basis of traditional phenotypic procedure as colonial, cellular morphology, microscopical examination and then streaking on MacConkey agar.

Biochemical reactions proved that all isolates were having the typical biochemical properties of *Pasteurella multocida*.

Serotyping of (15) Pasteurella isolates by using ELISA revealed that only 13(86.7%) out of 15 Pasteurella isolates were positive for ELISA.

The capsular serotyping of the selected (13) *Pasteurella multocida* isolates by using latex agglutination test were found belonging capsular serotype (A).

The somatic serotype 3 was the most prevalent one with a percentage of (53.3%) followed by serotype 12 with a percentage of (26.7%) and then serotype 1 with a percentage of (6.7%) by using the same test.

The recovered isolates were submitted for molecular identification of KMT1 gene (species specific gene for *Pasteurella multocida*) by PCR and found that only 14 (93.3%) out of (15) isolates were positive to KMT1 gene so confirmed to be *Pasteurella multocida*.

The incidence of *P. multocida* according to the age susceptibility was the highest at age 4-8 weeks and after weaning, older rabbits than 5 months to one year showing lower incidence mostly adult rabbits are believed to be carriers.

Studying of the immune status of rabbits to determine the comparative efficacy of our locally prepared bacterin and commercial bacterin in rabbits was carried out. The rabbits were vaccinated at 6 weeks of age and booster dose was given at 9 weeks. The seroconversion of antibody titer was estimated by PHA test which revealed significant difference at $P < 0.05$ when compared among prevaccination, primary vaccination and booster vaccination PHA titer in A and B group but between two groups (the locally prepared bacterin and the commercial one) and there is no significant difference.

The potency of vaccination was evaluated and determined by challenging of both vaccinated and control groups of rabbits with 1.0ml of (2×10^8) CFU of *P.multocida* isolates by S/C route at 21days post primary and post booster vaccination.

Results of challenge exposure demonstrated that:-

1. The oil-adjuvant rabbit pasteurellosis bacterins conferred excellent protection of vaccinated animals in all groups while all the unvaccinated control rabbits were infected and died following challenge infection except unchallenged group.
2. There was significant difference in PHA titer at $p<0.05$ after post primary and post booster vaccination during the period of observation, This study reported decrease of antibody titers after the challenge stage and this explain occurrence of antibody neutralization with bacterial

infection and presence of antibodies in dependable immune status in a level away from antibody titer of control rabbits at mean of 2.857± 0.4.

Referring to post challenge observations, control rabbits showed characteristic clinical signs and lesions like those of *rabbit pasteurellosis*.

Recommendations for Control and Prevention

This will focus mainly on control in breeding, laboratory and commercial rabbit colonies, which are most severely affected by the disease.

1- 1-**Good husbandry** is an essential part of disease control. Stress, intercurrent disease, poor air quality and overcrowding can trigger the flare up of latent infections. A clean, dry, well-ventilated environment is required with no draughts. Rabbits can withstand cold better than heat, and fluctuations in temperature should be avoided with a temperature maintained at around23-28°c.

2- Affected cases should be isolated and treated or culled promptly.

3- Keeping batches separate and minimizing contact between groups reduces disease transmission.

4- Disease free status can be achieved in colonies through barrier-housing and quarantine of any new stock.

5- Rabbits are placed in isolation for 2-4 weeks and multiple samples from their nasal cavities are submitted for culture.

6- Only rabbits with negative cultures are then used for breeding.

7- Serology and PCR can also be used to detect disease-free stock.

8- Antibiotics have been used prophylactically to prevent *Pasteurella* infection by administering them in the feed or water of pregnant does.

9- There appears to be a genetic resistance to *Pasteurella* infection and attempts have been made to produce disease-free strains of rabbits.

10- Vaccines are still being developed but protection seems to be incomplete and results have been disappointing, but still the oily

adjuvant bacterin proved to give higher immune response against disease especially when given as second poster dose after the formalized one by 14 to 21 days, as prevent mortalities; appearance of clinical signs of disease and also prevents or decreases shedding of *P.multocida* to unvaccinated animals.

11- The experimentally prepared oil adjuvant bacterin and commercial bacterin were safe and effective for the vaccination of rabbits against rabbit pasteurellosis with dual dose.

12- The vaccine produced better immune response especially, when booster dose was given after 21days of the primary dose.

13- We recommended that vaccination against rabbit pasteurellosis must be applied earlier than 6wks as infection may occur in ages earlier than 6 wks Especially in endemic areas.