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INVESTIGATIONS ON THE CARRIER RATE OF *PASTEURELLA MULTOCIDA* IN BLACK RATS (*RATTUS RATTUS*) IN A COMMERCIAL QUAIL FARM

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Abstract

The aim was to investigate the level of *Pasteurella multocida* infection from two anatomic sites of black rats (*Rattus rattus*), popularly referred to as house or roof rats in a commercial quail farmhouse with recurrent fowl cholera outbreaks and also to evaluate the association between the *P. multocida* found in rats co-habiting quail poultry houses and isolates from outbreaks of fowl cholera. Thus 100 pharyngeal and 100 rectum swabs samples taken from rats co-habiting farmhouse were obtained and evaluated bacteriologically for isolation of *P. multocida*; 54% of pharyngeal swabs and 62% of rectum swabs were positive for *P. multocida*. Extended phenotypic characterization of the isolates confirmed the presence of subspecies *P. multocida multocida*. Subspecies *P. multocida septica* and *gallicida* were not encountered. Random serotyping of 5 isolates each from the two sites confirmed serotypes A:4. Fowl cholera outbreaks were confirmed on the quail houses and carrier rats had the same *P. multocida* subspecies and serotype as the infected quail. The public health significance of the finding is also discussed.

INTRODUCTION

Bacteria included in the genus *Pasteurella* (family Pasteurellaceae) are commensals and occasional pathogens of many species of domestic and wild animals. Since first isolated by Pasteur as the causative agent of fowl cholera in 1880, the genus *Pasteurella* has

undergone many taxonomical and nomenclatural fluxes (1). Until recently, the genus included species formerly known as *Pasteurella pestis*, *P. pseudotuberculosis*, *P. enterocolitica*, *P. tularensis*, and *P. novicida*. The first three now constitute the genus *Yersinia* (family Enterobacteriaceae). The two

remaining species, the tularemia agents, have been given the generic name *Francisella*. (2).

Nearly half a century after the first isolation of *Pasteurella*, bacteria with common biochemical and morphological features were grouped together as *Pasteurella multocida*. Diagnosis of pasteurellosis depends on clinical appearance, and results of culture on blood agar. Colonies are small, grayish and non-hemolytic. *P. multocida* are small non-motile, Gram-negative cocobacilli often exhibiting bipolar staining, oxidase- and indole-positive. *P. multocida* strains are currently classified into 5 serogroups (A, B, D, E and F) based on capsular antigens and further classified into 16 serotypes based on lipopolysaccharide antigens (1). Despite serological similarities, *P. multocida* species is subdivided into three established subspecies: subsp. *multocida* subsp. *septica* and subsp. *gallicida* (3). A fourth subspecies (*tigris*) associated with tiger bites was recently proposed (1).

Many species of birds and mammals, including human beings, are susceptible to *P. multocida* infections. *P. multocida* has a global distribution and the organism is a normal flora of the upper respiratory and gastrointestinal tracts in a number of animal species. In cattle, sheep, goats, pigs, rabbits as well as domesticated and feral birds, *P. multocida* causes a life-threatening pneumonia and septicemia. In humans (especially the immunocompromised) who come in contact with animals with *P. multocida* infection, septicemia and chronic abscesses characterized by extensive edema and fibrosis may result (4, 5).

Fowl cholera caused by specific serotypes of *P. multocida* is a cause of concern in poultry industry where it causes economic losses in the form of death, treatment cost and labor. Official registrations of clinical disease outbreaks underestimate the prevalence of fowl cholera in Nigeria, although available records indicate that the disease is a major constraint in the increased production of poultry

chickens, turkeys, ducks, geese and quail in Nigeria. Outbreaks may result in very high mortalities of up to 80% (6 - 9).

A major issue for control of *P. multocida* infection in poultry flocks is the tendency to have reservoir or long-term carriers that periodically shed bacteria to the environment and contributes to the spread of infection within flocks. Carriers are animals that after initial infection continue to have the infection in internal organs and either continuously or intermittently shed high numbers of the organism through feces, aerosol etc. Vermin are known to be a significant reservoir for *P. multocida* causing fowl cholera (4). In poultry houses the most studied reservoir of *P. multocida* is the common brown rat (*Rattus norvegicus*) found predominantly in cooler regions notably Europe and North America. The black rat found in tropical countries are much less studied. With an estimated 10 million rat population in the world, Wincewicz (10) described rats as belonging to the most troublesome and detested plaques

tormenting people from the beginning of mankind. The economic damages inflicted on the human and animals by rats are mainly caused by their feeding routines and the serious hazard which results from the undisputed role in the epizootiology of many infectious and invasive diseases. The role of the brown rat in the epidemiology of fowl cholera in chicken and turkey farms is well documented (11, 12). This investigation reports the role of the black farm rat (*Rattus rattus*) which is largely confined to warmer areas of Asia and Africa, in the epidemiology of *P. multocida* infection in a commercial quail farm in north central Nigeria. The public health significance of the findings is also highlighted.

Materials and Methods

Rats

The black rats were obtained from the farmhouse by clubbing them to death. The killed rats were either processed immediately or kept in the deep freezer for not more than 3 days before processing. The rats were often found in dark gaps of building seats, in wall

corners, and in warm cellars. It is omnivorous and active 24 hours a day. It feeds everywhere: at cereal store or poultry houses, in waste dumps and shop store houses. Generally, it feeds on people's and animals food as well as on plants and small vertebrates.

Farm

Over a 6-month period, rats inhabiting two rooms housing flocks of approximately 90,000 commercial Japanese quail (*Coturnix coturnix japoninica*) on a well-managed farm were chased and clubbed down by farm workers. The quail flocks were usually housed in cement buildings with concrete floors covered with wood shavings and the quail were reared on the floor with feeds and water provided in troughs. Over the years (1999 - 2005), episodic outbreaks of pasteurellosis have been reported in the flock and also at the time of collection of these rat carcasses.

Phenotypic Examination

The killed rat heads were skin-shaven and the snouts aseptically snipped transversely with forceps to expose the

nasal cavity from which swab samples were collected from nasal turbinate and mucosa and plated onto blood agar (Oxoid) for overnight culture at 37°C. Each rat was equally swab-sampled from the inner rectal mucosa for similar isolation of bacteria. *Pasteurella*-like colonies on blood agar were selected for identification, subspeciation (3), and in some cases, serotyping (13).

Results

***P. multocida* isolation**

Of the 100 rat carcasses investigated for both pharyngeal and rectal isolation of *P. multocida*, 54 of the pharyngeal samples were positive, and 62 for the rectal samples obtained (Table 1).

Subspeciation

Some physiological characteristics are employed to differentiate *P. multocida* isolates into the various subspecies. The ability of the isolates to ferment mannitol, dulcitol and sorbitol was used to differentiate the subspecies (*Pasteurella multocida multocida*, *P. m. septica* or *P.m. gallicida*). All the isolates were positive for mannitol and sorbitol but negative for dulcitol - a reaction typical of *P. m. multocida*

Table 1: *P. multocida* isolation from two anatomic sites of farm rats (*Rattus rattus*)

Sample source	No. of sample	No. positive (%)
Nasal	100	54
Rectal	100	62

Serotyping

Indirect capsular-typing of five randomly picked *P. m. multocida* isolates was performed by using the hyaluronidase decapsulation test (13), and somatic serotyping of the isolates was performed by using the technique of Heddleston et al. (14). These random isolates were identified as *P.m.multocida* serotype A:4

Discussion

P. multocida is difficult to eradicate partly because of the presence of wildlife reservoir or alternative reservoir. *P. multocida* infection in population of black rats in this study showed a relatively high (over 50%) level of carrier rate when compared to other similar investigations (11, 12). However there was little in variation in the results from nasal to rectal samples obtained in this investigation.

P. multocida has been recovered from the oropharynx of wild Norway rats in Baltimore (USA) and from the nasal passages of wild rats obtained from poultry farms with pasteurellosis (12). These results and those of Curtis et al. (11) emphasize that nasal cultures alone are apt to yield results that underestimate the incidence of infection. The prevalence of *P.multocida* in the nares of rats ranged from 38 to 43% according to past surveys by other workers. In the assessment/evaluation of the sensitivity and specificity of techniques employed, Curtis et al (8) reported that 41% of samples taken proved positive after mouse inoculation, compared to 14% using media alone. Against this backdrop more positive samples may have been encountered if mouse inoculation had also been employed in this investigation.

Evidence of rat mortality due to pasteurellosis was not observed in our study and it has been reported by Manning et al. (12) that rats in contact with fowl cholera do not develop acute

infection but may become carriers of *P. multocida*. Although reports on naturally-occurring clinical infections in rats are lacking, or have received limited scientific study, anecdotal evidence suggest that *P. multocida* had produced pathological evidence of haemorrhagic septicaemia in affected organs (12), and Roberts and Gregory (15) also reported an epidemic of ophthalmitis due to *P. multocida* in hooded Lister rats.

The subspecies and serotype found in the rats correlate with those that have frequently been isolated from outbreaks of fowl cholera in the quail flock (9), lending credence to the role of the rats in the epidemiology of the episodic outbreaks often reported in the quail farm. This hypothesis is further substantiated by the fact that the disease was more frequent during the wet season and the cold harmattan period when rats migrated into buildings for warmth.

From a public health perspective, *P. multocida* is being added to the list of infective agents that are potentially

transmitted after an animal contact. Although statistics in Nigeria is lacking, it is documented that *P. multocida* is estimated to infect 20% - 50% of the 1-2 million Americans (primarily children) who are bitten or scratched each year by a variety of animals including dogs, cats, pigs, rats, lions, opossums and rabbits (16). Just as it has been estimated that as many as 66% of dogs and 90% of cats are colonized with this organism, typically in the respiratory and gastrointestinal tracts, our study also depicts a high percentage carriage of this organism in both tracts of rats. Persons at risk for infection related to animal exposure include veterinarians, farmers, livestock handlers, pet owners and food handlers. Immuno-compromised patients should be alerted about the potential risk related to poultry and laboratory small animal and pet animal contact, even when animals are in apparent good health.

The isolation from pharyngeal and rectal regions means that the organism could be transferred via aerosol and feces deposited on troughs containing

food or water. This study verified that aside from *Rattus norvegicus* species of rat, *Rattus rattus* has been incriminated as carrier of *P.multocida* and the rodent poses a major concern at the present time because of the readily transmission of *Pasteurella* spp from them to poultry.

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