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SUSCEPTIBILITY OF LABORATORY RODENTS AND CHICKS TO COLONIZATION WITH *GARDNERELLA VAGINALIS*

Nwaziri AA[1], Ezeifeka GO[2] and *Amadi ES[3]
[1]Department of Medical Laboratory Sciences, Faculty of Health Science and Technology, University of Nigeria, Enugu Campus, Nigeria
[2]Department of Applied Microbiology and Brewing, Faculty of Natural Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria
[3]Department of Applied Microbiology, Faculty of Applied and Natural Sciences, Ebonyi State University, Abakaliki, Nigeria

ABSTRACT

The present study investigated the susceptibility of rodents and chicks to *Gardnerella vaginalis* colonization. A total of seventy five rodents including albino rats, guinea pigs and rabbits were inoculated intra-vaginally and intra-muscularly respectively with *G. vaginlis*. Also, thirty day old and two week old chicks were inoculated intra-rectally and intra-muscularly. The animals were observed for three weeks. The respective rectal swabs were cultured and screened for *G. vaginalis* while the sera were examined for *G. vaginalis* antibody using agglutination and complement fixation tests. The rectal temperatures were determined. The vital organs including kidney, liver, heart, vagina, uterus, rectum and spleen were also screened for *G. vaginalis* colonization. The result of the study indicated *G. vaginalis* colonization in the vital organs of the rats and chicks inoculated with *G. vaginalis* (10^2-10^5 CFU/mL). Anti *G. vaginalis* antibody were detected in all the rodents and chicks inoculated. No colonization however was observed in the organs of guinea pigs and rabbits inoculated with *G. vaginalis*. This study therefore showed that albino rats and chicks are susceptible to *G. vaginalis* colonization and could serve as laboratory models for the study of *G. vaginalis*. However, the implication of this susceptibility with regard to the epizootiology of *G. vaginalis* associated diseases remain to be determined.

Keywords: *Gardnerella vaginalis*, rodents, colonization.

INTRODUCTION

Gardenerella vaginalis, formerly referred to as *Haemophilus vaginalis* (Gardner and Duke, 1954), is a facultative, chemoorganoheterotrophic, oxidase negative, catalase negative, pleomorphic, gram negative or gram variable bacilli or coccobacilli (Singleton, 1999). Prior to the discovery of *G. vaginalis*, women that had malodorous vaginal discharge, not associated with Trichomoniasis, Candidiasis or Gonorrhoea were said to be having non-specific vaginitis or bacterial vaginosis (Gardner and Duke 1954; Holmes *et al*., 1981). Although, bacterial vaginosis (BV) is a multi-microbial disease, *G. vaginosis* has been discovered in numerous quantities among women suffering from BV (Spiegel *et al*., 1980; Piot *et al*., 1982). Some workers have associated the etiology of BV to *G. vaginalis* (Demba *et al*., 2005). *G. vaginalis* was originally not associated with serious complications, but recent reports indicates that they are involved in the etiology of numerous upper genital tract diseases and conditions (Kimbalin and Andrew, 1998).

Although reports on the colonization of rodents and birds by *G. vaginalis* are uncommon in Nigeria, a number of studies in the western countries have indicated its isolation from Fox, Racoon dogs, Mare and Mink (Yan *et al*., 1995; Salmon *et al*., 1990). The association of *G. vaginalis* with rodents and chicks may be of strategic epidemiological concern. The present study intends to assess the susceptibility of laboratory rodents and chicks to *G. vaginalis* colonization with a view to highlighting the possible epizootiological importance.

*Corresponding author email: amadies2001@yahoo.com

MATERIALS AND METHODS

Gardnerella vaginalis isolates :

Pure cultures of *G. vaginalis* isolated from bacterial vaginosis patients were obtained from the Medical Microbiology laboratory, University of Nigeria Teaching hospital, Enugu, Enugu State, Nigeria. The isolates were confirmed using standard methods (Collins *et al*., 1995; Cowan, 1974).

Selection of Laboratory animals and chicks

Three rodents including albino rats, guinea pigs and rabbits were used. In each case, twenty five female species were randomly selected from the animal house of the University of Nigeria Teaching hospital, Enugu. Fifteen day-old and fifteen two-week old chicks were randomly selected. They were fed on commercial feed

Table 1. Organ distribution of *G. vaginalis* in rats inoculated intramuscularly with 0.02ml of 10^5 CFU/mL of *G. vaginalis.*

Organs Colonized	Test Rats										Control
	1	2	3	4	5	6	7	8	9	10	
Kidney	$>10^5$	10^5	10^5	10^5	10^5	10^5	10^5	10^5	10^5	10^5	NIL
Heart	10^4	10^4	10^4	10^2	10^2	10^2	10^2	10^2	10^2	10^2	NIL
Liver	10^2	10^2	10^2	10^2	10^2	10^2	10^2	10^2	10^2	10^2	NIL
Spleen	10^2	$<10^2$	10^2	10^2	$<10^2$	10^2	10^2	10^2	10^2	10^2	NIL
Blood	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL

Key: $10^5 = 10^5$ CFU/ unit mass of the organ, $10^4 = 10^4$ CFU/ unit mass of the organ, $10^2 = 10^2$ CFU/ unit mass of the organ.

Table 2. Spread of infection after intra-vaginal inoculation of 0.02ml of 10^5CFU/mL of *G. vaginalis* in rats.

Organs Colonized	Test Rats										Control
	1	2	3	4	5	6	7	8	9	10	
Vagina	$>10^5$	$>10^5$	10^5	10^5	10^5	10^5	10^5	10^5	10^5	10^5	NIL
Uterus	10^4	10^2	$<10^2$	NIL	10^2	NIL	10^2	10^2	10^2	$<10^2$	NIL
Heart	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
Liver	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
Spleen	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
Blood	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL

Key: $10^5 = 10^5$ CFU/ unit mass of the organ, $10^4 = 10^4$ CFU/ unit mass of the organ, $10^2 = 10^2$ CFU/ unit mass of the organ

Table 3. Antibody formation in 3 weeks old albino rats inoculated by various routes with 0.02ml Containing 10^5CFU/mL of *G. vaginalis.*

	Route of Inoc.	*G. vaginalis* dose (ml)	Agglutination Test titre range	CFT Titre range
Test	Intramuscular	0.02	1:20 – 1:80	1:20 – 1:80
Rats	Intravaginal	0.02	1:5 – 1:40	1:5 – 1:40
Control	Intramuscular	-	No antibodies	No antibodies
Rats	Intravaginal	-	No antibodies	No antibodies

and observed for four days to ensure that they were healthy before the commencement of the study.

Inoculation of Laboratory animals and chicks with *G. vaginalis*

Ten laboratory animals of each species were inoculated intra-vaginally and ten intra-muscularly with 0.02 ml saline suspension of *G. vaginalis* contining 10^5 CFU/mL. The remaining five animals in each served as controls without *G. vaginalis* inoculation. The control was inoculated with 0.02ml of sterile saline containing no organism.

Ten day-old and ten two-week old chicks were inoculated intra-cloacally with the same quantity of *G. vaginalis*. Five chicks in each case served as controls. All the experimental animals were kept under close observation for three weeks.

Determination of Rectal Temperature

The rectal temperatures of the respective animals were determined before inoculation and after 7^{th}, 14^{th} and 21^{st} days post inoculation using clinical thermometer.

Cultivation of Swab samples

Vaginal swabs of the respective rodents and the cloacal swabs of the chicks were obtained using sterile swabs and then inoculated into Brain Heart infusion agar supplemented with 5% blood, 4mg/l genticin and 32mg/l Nalidixic acid. The plates were incubated anaerobically at 37°C. The isolates were screened for *G. vaginalis* using standard methods (Collins *et al*., 1995; Cowan, 1974). This was done before and after the experimental period.

Serological Screening for *G. vaginalis* antibody

Serum samples collected before and at the end of the experiment from all the animals were tested for the presence of *G. vaginalis* antibody by the agglutination and complement fixation tests (Stokes, 1970; Cheesbrough, 1994).

Detection of Clue cells

The presence of clue cells in the vaginal and/ rectal swabs of the animals before and at the end of the experiment were determined using standard methods (Cheesbrough, 1994; Lo *et al*., 1997).

Table 4. Antibody formation in 2 months old rabbits inoculated by various routes with *G. vaginalis.*

	Route of Inoc.	*G. vaginalis* dose(ml) Agglutination	CFT Titre range	Test titre range
Test	Intramuscular	0.02	1:5 – 1:20	1:5 – 1:20
Rabbit	Intravaginal	0.02	No antibody	No antibody
Control	No inoculation	-	No antibody	No antibody

Table 5. Antibody formation in 2 months old Guinea pigs inoculated by various routes with *G. vaginalis.*

	Route of Inoc.	*G. vaginalis* dose(ml)	Agglutination Test titre range	CFT Titre range
Test	Intramuscular	0.02	1:5 – 1:40	1:5 – 1:40
Rabbits	Intravaginal	0.02	No antibody	No antibody
Control	No inoculation	-	No antibody	No antibody

Table 6. Colonization of the organs of chicks by *G. vaginalis* inoculated via rectal route.

Organs Colonized	Test Chicks										Control
	1	2	3	4	5	6	7	8	9	10	
Kidney	10^2	10^4	10^4	10^4	10^4	10^4	10^2	10^2	10^2	10^4	NIL
Heart	10^2	10^2	10^2	10^2	10^2	10^2	10^2	10^2	10^2	10^2	NIL
Liver	10^2	$<10^2$	$<10^2$	10^2	10^2	$<10^2$	$<10^2$	$<10^2$	NIL	NIL	NIL
Lungs	$<10^2$	NIL	$<10^2$	NIL	NIL	NIL	10^2	10^2	10^2	10^2	NIL
Uterus	10^2	10^2	10^2	10^2	10^2	10^2	10^2	10^2	10^2	10^2	NIL
Rectum	10^5	10^5	$>10^5$	10^5	10^5	10^5	10^5	10^5	10^5	10^5	NIL

Key: $10^5 = 10^5$ CFU/ unit mass of the organ, $10^4 = 10^4$ CFU/ unit mass of the organ, $10^2 = 10^2$ CFU/ unit mass of the organ

RESULTS AND DISCUSSION

The result of the study showed that clue cells and *Gardnerella vaginalis* were recovered from the vaginal and cloacal swabs samples obtained from albino rats and chicks infected with *G. vaginalis* after 21 days. Also, *G. vaginalis* colonized their vital organs including kidney, liver, heart and spleen at the end of the experimental period (Tables 1, 2, 6). Previous study had reported that ten pig tailed Macaques inoculated intra-vaginally with *G. vaginalis* were colonized for 11-39 days (Johnson *et al*., 1984). Although the apparent variation in the vaginal microenvironment of rats and humans, may limit the use of rats as a perfect model for studying the infections in humans, the finding in this work indicates that rats could probably be useful in study of the biology and immunology of *G. vaginalis*.

The susceptibility of *G. vaginalis* has also been reported in Fox, Racoon dog, Mink and Canine (Salmon *et al*., 1990; Yan *et al*., 1995; Yan *et al*., 1996). 145 strains of *G vaginalis* was isolated from Foxes from 13 main farms in six provinces of China (Yan *et al*., 1996). The colonization was however found to be more among the albino rats inoculated via the intra-muscular route than in others. This may not be unconnected with the fact that intra-muscular injection leads to quicker access to the blood circulation than the vaginal route.

Although information on the infection of *G. vaginalis* in birds including chicks are generally scarce, their susceptibility to other bacteria infections has been reported. *Escherichia coli* infection for instance, has been described in layer hens, turkeys, geese and ducks causing significant economic loss (Landman and Cornelissen, 2006). Infection of poultry to Salmonella gallinarum-pallorum and Campylobacter has also been reported (Johnson *et al.*, 1977; Sato *et al*., 1997; Erbeck *et al*., 1993). It is vital to note, that in this study all the day-old chicks inoculated with *G. vaginalis* had rigor/restlessness, weight loss and retarded growth. 80% of them died before the three weeks period. Although, the two-week old chicks similarly inoculated, had reduced level of clinical signs, 40% of them also died at the end of 21 days. It is therefore most likely that, besides infection and colonization of vital organs of chicks, *G. vaginalis* may be implicated in the etiology of poultry diseases, going by the observed clinical signs and deaths. Further studies may be necessary to determine the nature of the poultry disease possibly introduced by the inoculation of *G. vaginalis*.

The susceptibility of the rats and chicks to *G. vaginalis* as determined in this study could have some epizootiological significance. This is particularly important to animal breeders and poultry farmers, who come close to animals and birds on daily basis. Previous research reported a 0.9-

21.9% *G. vaginalis* infection rate in foxes which resulted in abortion rate of 1.5-14.7 % (Yan *et al*., 1995). The workers further stated that, *G. vaginalis* disease of Fox were able to infect their feeders and farm manager. This calls for some precaution by animal breeders and poultry farmers to avert possible zoonosis.

This study also indicated that guinea pigs and rabbits were not susceptible to *G. vaginalis* colonization after 21 days. This is consistent with the work of Yan and colleagues (1995) in which mouse, big white rat, gopher, guinea pig and rabbits were not infected with *G. vaginalis*. Prio study also asserted that 4 tamarins and 3 chimpanzees inoculated with *G. vaginalis* failed to become colonized (Johnson *et al*., 1984).

The intra-muscular and intra-vaginal inoculation of the rats with *G. vaginalis* led to the production of high level of antibody with titre range 1:5 to 1:80 (Table 3), while the intra-muscular inoculation of guinea pigs and rabbits yielded titre level of 1:5 to 1:40 (Table 4, 5). The antibody levels produced in the inoculated chicks were generally 1:5 titre. Although this confirms the immunogenicity of *G. vaginalis*, the antibody titre recorded for day old and 2-week old chicks appear to be non-protective. This may have accounted for the high levels of mortality recorded.

While recommending the use of albino rats and chicks for future biological and immunological studies of *G. vaginalis*, further investigation is hereby suggested to determine the nature of disease possibly induced in these species.

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