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Full Length Research Article

Environmental Bacteria Associated With an Institutional Rabbit House

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ABSTRACT

A bacteriological investigation of microorganisms of public health importance associated with rabbit houses was undertaken to determine the occurrence of bacteria in rabbit house in Ibadan. A total of 144 swab samples were collected from which 160 bacterial isolates were recovered. *E. coli*, (20%) showed the highest occurrence, followed in descending order by *Staphylococcus aureus* (12.5%), *Proteus vulgaris* (12.5%), *Bacillus cereus* (12.5%), *Bacillus subtilis* (10%), *Streptococcus faecalis* (10%), *Bacillus firmus* (*7.5%*), *Proteus morganii* (5%), *Pseudomonas aureginosa* (I2.5%), *Streptococcus pyogenes* (2.5%), *Micrococcus* species (2.5%) and *Klebsiella* species (2.5%). All the 20 (12.5%) staphylococcal isolates were coagulase-positive using tube coagulase test with human plasma. Similar strains of bacteria encountered in this investigation have been incriminated in disease outbreaks in rabbits with losses in terms of meat meant for human consumption and are therefore of public health importance. There is the need for regular microbiological surveillance to protect our growing rabbitaries and the rabbit models in biomedical research since these latent organisms may produce clinical conditions when the rabbits are exposed to stress conditions. Above all the importance of good hygiene and management in rabbitaries cannot be overemphasized to prevent avoidable outbreaks.

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INTRODUCTION

The high cost of protein and rapid increasing population in developing countries, are the major constraints militating against the availability of the much-needed high quality protein food to low income earners. This situation calls for the production of fast maturing animals like rabbits with the utilization of cheap and locally available feedstuff in order to produce them at an affordable cost (Bale and Iyeghe-Erakpotobor, 2003)

However, the protection of consumers from disease-causing microorganisms through regular microbial surveillance and hygiene thereby reducing their contamination of foods is now of International concern. The health of animals and food safety factors, have led to technical barriers against International food trade. In some instances the effects of microbial agents are more insidious (Lipman and Perkins, 2002). Approximately one-quarter of global disease burden is due to environmental factors (W.H.O., 2006). Animal health hazards among other factors may be due to soil – borne hazards which arise as a result of the transmission of spores, which originate from infected excretions, or the remains of sick or dead animals developed on or in the soil (Seifert, 1996).

In an earlier investigation, Ajuwape and Aregbesola (2002) found 108 aerobes and recorded 100% incidence of coagulase-positive *Staphylococcus aureus,* followed by *Klebsiella pneumonia* (10%), *Micrococcus luteus* (9%), *Escherichia coli* (6%), *Streptococcus zooepidemicus* (4%) and *Pseudomonas aeruginosa* (1%) from the respiratory of rabbits in Nigeria. Recently, Ajuwape *et al.,* (2006) reported the occurrence of the following bacteria: *Staphylococcus aureus,* (53.2%), followed by *Staphylococcus epidermidis* and *Streptococcus zooepidermicus* with (12.9%) from ear canals domestic rabbits. Other microbes encountered were *Escherichia coli* with an occurrence 8.1% and *Micrococcus luteus* (6.5 %). *Streptococcus morbillorum* and *Pasteurella multocida* showed an occurrence of 3.2 % respectively.

The current investigation was undertaken to determine the prevalence of microbes of public health importance associated with rabbit house that constitute the immediate environment of the rabbits.

MATERIALS AND METHODS

Environmental fittings sampled: Using sterile swabs moistened with Trypticase soy broth (TSB) (BBL, Becton Dickonson, Cockeysville, Md.) samples were obtained from Institutional Rabbitry of the Institute of Agricultural Research and Training, Moor Plantation Ibadan, Oyo State, Nigeria. Swab samples were collected from ten (10) different sites in each rabbitary: the water troughs, inner house drainage (left and right sides), main water source, outer house drainage (left side and right), nesting boxes, outside window, main entrance door, feed. Using sterile forceps, rat faecal droppings from were collected from the rabbitary and sterile TSB was used as control. Four replicates of each sample was collected.

Culturing Techniques: Briefly, 1.0 g suspension of rat faecal droppings was placed in 9.0ml of peptone water and vortexed. After which 0.1ml of the same buffer spread on blood agar (2 plates) and MacConkey agar. Also the sterile swabs were inoculated onto TSB and incubated overnight at 37^{o}C. Four replicates of each specimen were collected as indicated above. The cloudy broths suggestive of growing microbes were inoculated onto onto sheep blood agar (Oxoid Columbia blood agar® CM331) and MacConkey agar No. 2 (Oxoid CM 109®) plates and were incubated aerobically at 37^{o}C for 24-72 hours. Another set of the blood agar plates were incubated anaerobically at 37^{o}C to detect strict anaerobes in the samples Haemolysis and pigmentation were scored after 24 hours. Following incubation, growth characteristics and colony morphology of the cultures were studied. The colonies were subjected to standard biochemical test procedures described by Barrow and Feltham, (1993).

Coagulase activity of Staphylococcal isolates: Using human plasma, colonies yielding Gram-positive cocci with catalase-positive and oxidase-negative reaction, were subjected to coagulase test

by tube method as follows. Two drops of overnight cultures in TSB (Trypticase Soy broth, Merck®, Germany) were added to test tubes containing 1.0 ml of freshly prepared 1:10 dilution of citrated human plasma in saline. The mixture was incubated at 37°C and examined every two hours for clot formation over a period of 24 hours (Langlois *et al.,* 1990).

RESULTS

A total of 160 bacteria isolates were recovered in the four replicates from the rabbit house. The highest prevalence was shown by *E. coli* 32 (20%), followed by *Staphylococcus aureus* 20(12.5%), *Proteus vulgaris* 20(12.5%), *Bacillus cereus*20(12.5%), *Pseudomonas aureginosa* 20(I2.5%), *Bacillus subtilis* (10%), *Streptococcus faecalis* (10%), *Bacillus firmus* (*7.5%*), *Proteus mirabilis* (5%), *Streptococcus pyogenes* (2.5%), *Micrococcus* species (2.5%) and *Klebsiella* species (2.5%) (Tables I and 2). All the 20 (12.5%) staphylococcal isolates were coagulase-positive using tube coagulase test with human plasma.

DISCUSSION

This investigation shows that *Escherichia coli* had the highest prevalence of 20% among the bacteria recovered from the rabbit environment studied. This is higher than those earlier reported by Ajuwape and Aregbesola, (2002) and Ajuwape *et al.,* (2006) from the respiratory tract and ear canal of rabbits respectively. The coliforms (*Escherichia coli* and *Klebsiella* species) encountered in this study were similarly observed as the most common environmental bacteria frequently incriminated in bovine mastitis by Jones, (1990). This high prevalence of *Escherichia coli* constitutes a potential source of infection to the rabbits since *E. coli* have associated with gut oedema and enteric colibacillosis which is manifested as diarrhoea and sudden death in rabbits (Adetosoye, 1984; Gross, 1991). Colibacillosis diarrhoea is also capable of producing exotoxin and endotoxin (Sussanana, 1985). While acute bacterial pneumonia of rabbit has been associated with *Klebesiella pneumonia* (Dhand *et al.,* 2001). *Escherichia coli,* was recovered from faecal samples of rats in the rabbitary.

Table 1:
Summary in Percentage of Bacterial Isolates

Microbe*	A	B	C	D	E	F	G	H	I	J	K
Escherichia coli	80	-	-	80	80	82	60	60	80	-	80
Staphylococus aureus	80	-	64	60	60	-	-	52	-	-	-
Proteus vulgarius	60	-	60	64	-	-	-	48	-	-	44
Bacillius cereus	-	-	60	48	40	-	-	-	-	60	64
Bacillus subtilis	-	-	42	-	-	36	48	-	60	-	-
Streptococcus faecalis	28	20	-	-	32	-	-	32	-	-	-
Bacillus firmus	-	-	-	-	-	32	52	-	-	36	-
Proteus mirabilis	-	40	-	-	32	-	-	-	-	-	-
Klebsiella species	-	-	36	-	-	-	-	-	-	-	-
Micrococcus species	40	-	-	-	-	-	-	-	-	-	-
Pseudomonas aureginosa	-	-	-	-	-	-	-	-	36	-	-
Streptococcus pyogenes	-	32	-	-	-	-	-	-	-	-	-

* No of replicates per sample (4) x by each identified organisms = Total No of the organism observed x by 100 = %

KEY:-

+ = Present - = Absent

A: Water trough; G: Swabs of nesting boxes; B: Left inner house drainage; H: Outside windows; C: Main water source; I: Main entrance door; D: Left outer house drainage; J: Feed sample: E: Right outer house drainage; K. Faecal sample of rat; F: Right inner house drainage

Table 2:
Percentage Bacteria Isolates Obtained From the Rabbitary

E. coli	32 (20%)
Staphylococcus aureus	20 (12.5%)
Proteus vulgaris	20 (12.5%)
Bacillus cereus	20 (12.5%)
Bacillus subtilis	16 (10%)
Streptococcus faecalis	16 (10%)
Bacillus firmus	12 (7.5%)
Proteus morganil	8 (5%)
Klebsiella species	4 (2.5%)
Micrococus species	4 (2.5%)
Pseudomonas aureginosa	4 (2.5%)
Streptococcus pyogenes	4 (2.5%)

The occurrence of *Staphylococcus aureus*, *Proteus vulgaricus* and *Bacillius aureus* (12.5%) each was next to *Escherichia coli*. Ajuwape and Aregbesola (2002) however reported the highest occurrence of *Staphylococcus aureus* in the upper respiratory of normal rabbits studied. All the *Staphylococcus aureus* encountered in this investigation were coagulase positive, this calls for concern because Devriese *et al*., (1978) and Ajuwape and Akinyede, (2001) reported a correlation between coagulase production and staphylococci pathogenicity. Hence, the isolates are potentially pathogenic. The management methods of the rabbitaries may influence the distribution of microbes in the environment as well as the flora of the various parts of the animals located in the environment. Staphylococcal infection results in rapidly evolving abscesses of rabbits (Devriese *et al,* 1996). Other clinical presentations earlier associated with staphylococcosis are sore hock (pododermatitis), pneumonia, metritis and abortion (Carolan, 1986, Ajuwape and Aregbesola, 2001).

In this investigation *Klebsiella* species showed 2.5% prevalence. *Klebsiella* species in rabbits exhibits pyrexia, sneezing, nasal discharge & respiratory distress (Dhand, *et, al*; 2001). The effect is more severe in young than in adult rabbits. High environmental temperature, high humidity and poor ventilation inside the rabbitary serve as other stress factors (Lipman and Perkins, 2002).

Streptococcal faecalis causes endogenous urinary tract infection in man, it is involved in the formation of dental caries and periodontal disease. Its high resistance to heat and many antimicrobial drugs makes treatment difficult. Its high prevalence is an indication of high environmental pollution of the colony (Lipman and Perkins, 2002).

This study shows that the rabbit house investigated habours bacteria and could be an important source for microbial dissemination. This is similar to the recent findings of Muirhead *et al*., (2006) that bacteria especially *E. coli* attached predominantly to small particles and remained unattenuated. The presence of rats on the farm from adjacent fields could be associated with an additional increased disease risk. This is especially so, because seven microorganisms were found in their faecal samples. As recommended recently by W.H.O., (2006) disease can be prevented through the environment. Hence, it is advocated that proper environmental sanitation and hygiene should be observed to effectively control or prevent avoidable disease outbreaks in rabbit houses.

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