SEMEN BACTERIAL FLORA OF RHODE ISLAND BREEDER COCKS IN ZARIA, KADUNA STATE, NIGERIA

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ABSTRACT

The semen used in this study was collected from 77 Rhode Island Breeder cocks reared in battery cages under intensive management from a private farm in Zaria, Kaduna State, Nigeria using the back massage procedure, 27 of the 77 semen samples (35.1%) contained bacterial isolates. None of the samples grew fungi. Bacteria isolates obtained from the semen include: *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Proteus species* and *Klebsiella species*. Seventy of the semen samples were negative for brucellosis but seven samples exhibited *Brucella* specie agglutinins using tube agglutination test and the level of antibody titres are 61.5, 82.0 and 102.5 iu/ml respectively. The presence of *Brucella* agglutinin detected in this study is significant since brucellosis is of public health and economic significance. In addition, the presence of bacteria contaminants in semen should be viewed with seriousness. As a consequence, routine control of bacteria in collected semen seems desirable. This study sought to identify the bacterial flora and pathogens in semen collected from cocks and see how they may be effectively reduced or destroyed in the interest of the efficient collection, preservation and delivery of highly fertile semen artificially. Areas for further investigations were highlighted.

Keywords: Cocks, Semen, Bacterial flora, Artificial Insemination, Rhode Island Breeder.

INTRODUCTION

Two of the advantages of artificial insemination (AI) in poultry are the reported increase in fertility and hatchability rates in turkeys and chickens (Lake, 1967; Clark *et al.*, 1982). Various investigators have conducted work on

semen production and quality on a variety of poultry breeds and strains. Saeid and AL-Soudi (1975) reported breed and seasonal differences in semen production of cocks, while Egbunike and Oluyemi (1979) showed that breed and time of semen collection affect cock semen. Omeje and Marire (1990) observed that significant genotype differences affected body size and semen characteristics of cocks, except the pH value, while age differences significantly affected variation in the body size, semen volume per ejaculate and pH value. In addition, age by genotype interaction effect was important only for semen volume.

Although extensive work has been done on AI in sheep (Osinowo, 1992), there is a dearth of information on the bacteriology of semen from this small ruminant. Similarly, there is a paucity of information on the bacterial flora of cock semen in northern Nigeria.

Semen is a good medium for growth of bacteria. Perhaps the first to report such an observation was Spallanzani, who in 1785, observed that the sperm from the terrestrial fetid toad soon became putrid. He attributed the diminishment of fertility of the sperm during storage to this putrefaction. Spallanzani's observations have long been confirmed variously by many seminal bacteriologists, (Roemmele, 1972).

As a matter of routine practice, workers in the rapidly expanding field of AI stress the need for bacteriological control in semen which is collected. Therefore it seems desirable to establish what measure of bacteriological control is required in the collection, handling, storage and transportation of semen. In addition, information on the types and numbers of bacteria obtained under varying conditions of collection and storage and their possible relation to infection in the female genital tract would be useful, thus justifying the purpose of this investigation.

MATERIALS AND METHODS

Cock Semen Collection:

The semen used in this study was collected from 77 Rhode Island Breeder cocks reared in battery cages under intensive management from a private farm in Zaria, Kaduna State, Nigeria using the back massage procedure (King, 1981; Lake et al., 1985). This involved cleaning the cloacal region with a damp, clean towel and then massaging the back of the male cock. The semen was gently squeezed into a sterile collecting vessel.

Bacteriological Examination of Semen:

Each semen sample collected was inoculated within 15 minutes after collection, using aseptic techniques, onto blood agar (BA, containing 5% sterile sheep blood), MacConkey agar (MAC), Salmonella-shigella agar (SSA), Cholera medium (TCBS), Farrell's medium (FM) and Sabourand dextrose agar (SDA). All media were prepared in accordance with manufacturer's instruction and standard bacteriological procedures (Cowan, 1981). The culture plates were incubated at 37°C

kept at room temperature for one hour to inhibit any bacterial growth. The mixture was then centrifuged at 1000 x G for 5 minutes. The supernatant (seminal plasma) was separated and subjected to the brucellosis tube agglutination test (Corbel et al., 1978). Seminal plasma was serially diluted 1:10, 1:20, serially to 1:80. The *Brucella abortus* antigen (from CVL, Weybridge, Surrey, England) was diluted 1:10 for use.

RESULTS

27 of the 77 semen samples (35.1%) contained bacterial isolates. None of samples grew fungi. The type of isolates and their number of isolations are shown in Table 1. The bacterial isolates are: *Escherichia coli*, *Klebsiella species*, *Staphylococcus aureus*, *Streptococcus faecalis* and *Proteus species*.

Seventy of the semen samples were negative for brucellosis but seven samples exhibited *Brucella* specie agglutinins and the level of antibody titres are 61.5, 82.0 and 102.5 iu/ml respectively (Table

TABLE 1: BACTERIAL ISOLATES AND FREQUENCY OF ISOLATION FROM TWENTY-SEVEN BACTERIA-POSITIVE SEMEN SAMPLES

Bacteria isolate	No isolate	<i>Escherichia coli</i>	<i>Klebsiella species</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus faecalis</i>	<i>Proteus species</i>
No. of isolates	50	8	5	2	10	4

TABLE 2: COCK SEMEN TUBE AGGLUTINATION TEST (SAT)

	SAT Negative		SAT Positive		
Dilution (iu/ml)	1:10 to 2:80 (23) (45)		2:40 (61.5)	4:40 (82.0)	1:80 (102.5)
	70		1	2	4

and observed daily for growth. One BA plate was cultured anaerobically and incubated at 37°C. Where there was no growth, all plates were incubated for one week before being designated negative. Cultures on SDA plates for fungi were incubated at room temperature (28 to 30°C) for 5 - 10 days. Biochemical tests were performed on all of the isolates using standard bacteriological techniques according to Macfaddin (1981).

Brucellosis Test On Semen:

To 0.5 ml of whole semen was added one drop of a one per cent solution of methiolate. These were

2).

DISCUSSION

The results obtained indicated that bacteria were present in semen collected from about one-third of the cocks semen examined. However, the bacteria isolated are not pathogenic but indicators of pollution or contamination from faecal droppings underneath the cages, litters and cages from where the cocks are housed. In addition, contaminations could come from handlers and improperly sterilized fomites. The isolates are less fastidious and would grow readily to preclude

some actual pathogens if special culture media are not used for isolation. This was taken care of in this study since special culture media were used to eliminate the less fastidious organisms. These bacteria can be easily eliminated or materially reduced in number with proper hygiene and strict aseptic control measures. Bale and Kumi-Diaka (1981) have also reported *micrococci*, haemolytic and non-haemolytic streptococci, *Pseudomonas* sp., unidentified rods, coliform organism and *Brucella abortus* from bull semen.

Pathogenic infections from bacteria and fungi (though not present in this study) should be viewed with seriousness since it could lead to functional and anatomical changes which might result in varying degrees of impotency (e.g. poor semen quality, infertility, sterility etc) which may likely hinder the use of infected cock semen for an artificial insemination programme.

The non recovery of *Brucella* organisms in this study may have been affected by the limited number of sample collected and restricted use of semen only for isolation. It is possible that isolations could have resulted from examination of a wider range of organs. This would need further investigation.

The high agglutinating antibody titres exhibited by seven semen samples is an indication of probable *Brucella* infection. The overall low serological result obtained may be due to the fact that agglutinating antibodies produced during initial stages of infection may not last long. Hence these may not be detectable in later stages while the precipitating antibodies take sometimes to develop after infection but remain for a long period or it may be as a result of cross-reaction of *Brucella* organisms with other bacteria organisms. Since a single test may not be capable of detecting all infected cases, supplementary tests may be used to rule out the possibility of false negatives. Bale and Nuru (1982), and Chukwu and Anene (1988) have demonstrated that brucella agglutinin is present in local and free-ranged fowl's sera in Nigeria. Efforts should be made to isolate, characterise and biotype *Brucella* and other pathogenic organisms from local and imported chickens in the country.

REFERENCES

- BALE, O.O.J. and KUMI-DIAKA, J. (1981). Serological and Bacteriological study of bovine brucellae from Livestock Investigation Centres in Nigeria. *British Veterinary Journal*. 137: 265 - 261.
- BALE, J. O. and NURU, S. (1982). Serological study of brucellosis in local fowls in Northern Nigeria. *Journal of Animal Production Research*. 2(1): 53 - 62.
- CLARKE, R.N., SEXTON, T.J. and OTTINGER, M.A. (1972). Effects of holding temperature and storage time on respiratory rate, motility, and fertility of chicken and turkey semen. *Poultry Science*. 61: 1912 - 1917.
- CHUKWU, C.C. and ANENE, B.M. (1988). Brucellosis in local chicken at Nsukka. *Zariya Veterinarian*. 3(1) 36 - 39.
- CORBEL, M.J., GILL, K.P.W. and THOMAS, E.L. (1978). Methods of identification of brucella. Report Series No. 2 Weybridge: Central Veterinary Laboratory.
- COWAN, S.T. (1981). Identification of Medical Bacteria. 2nd ed. Cambridge University Press, London, New York, Sydney.
- EGBUNIKE, C.N. and OLUYEMI, J.A. (1979). Comparative studies of the reproductive capacity of the Nigerian and Exotic poultry breeds. *Nigerian Journal of Animal Production*. 6: 47 - 51.
- KING, A.S. (1981). Phallus. In: Form and Function in Birds. Ed. by A.S. King and J. McHolland Vol. 2: 107 - 147. London Academic Press.
- LAKE, P.E. (1962). Artificial insemination in poultry. In: Maile, J.P. (eds.) The semen of animals and artificial insemination. Commonwealth Agriculture Bureau, Bucks, England, pp. 331 - 335.
- LAKE, P.E., RAVIE, O. and WADDINGTON, D. (1985). Some effects of the composition of inseminated semen and the site of its deposition and fertility in *Gallus domesticus*. *Animal Reproduction Science*. 9: 273 - 284.
- MACFADDIN, J.P. (1981). Biochemical tests for identification of medical Bacteria 2nd ed. Williams and Wilkins, Baltimore, London.
- OMEJE, S.S.I. and MARIRE, B.N. (1990). Evaluation of semen characteristics of adult cocks of different genetic background. *Theriogenology*. 34: 1111 - 1116.
- OSINOWO, O.A. (1992). Sheep artificial insemination research at NAPRI: 1979 - 1992. NAPRI Seminar Paper, Shika. Presented on Nov., 3, 1992.
- ROEMMELE, O. (1972). Biologische und physiologische untersuchungen an scheroensckret des Rindes in Hinblik auf die kunstliche Besamung. *Zool. Jahrb. Abt.-F. Allg. Zool. U-physiol*. 44: 85 - 148.
- SAEID, J.M. and AL-SOUDI, K.A. 1975. Seasonal Variation in semen characteristics of White Leghorn, New Hampshire and indigenous chickens in Iran. *British Poultry Science*. 16: 91 - 102.

BALE, O.O.J. and KUMI-DIAKA, J. (1981). Serological