Comparison between Fluorescent In Situ Hybridization (FISH) and Culture Method in the Detection of Pasteurella Multocida in Organs of Indigenous Birds

Mbuthia, P.G.1, Njagi L.W.1, Nyaga P.N.1, Bihora L.C.1, Mugera G.M.1, Kamundia J.1, Minga U.1, and Olsen J.E.4
1 Department of Veterinary Pathology, Microbiology and Parasitology, University of Nairobi, P.O. Box 29053, Nairobi, Kenya.
2 Plant Breeding Station, Kenya Agricultural Research Institute, P.O. Njoro, Kenya.
3 Department of Microbiology and Parasitology, Sokone University of Agriculture, P.O. Box 2021, Morogoro, Tanzania.
4 Department of Veterinary Pathology, The Royal Veterinary and Agricultural University, DK – 1870 Frederiksberg C., Denmark.

Abstract
A total of forty-eight indigenous birds were intratracheally infected with Pasteurella multocida, paired and sacrificed at specified times. Seven organs from each of the four pairs were swabbed for culture and tissues taken for FISH test to detect the presence of the bacterium in these birds. Oropharyngeal and cloacal swabs were collected, for culture method and bacteria characterized by biochemical tests. While for FISH test, tissues were processed for histology after fixation in formalin for 24 hours and later preserved in 70% alcohol before in situ hybridization test. At any sacrificial time between 0 hour and 14 days post inoculation P. multocida FISH signals were observed in 47 to 73% while the bacterium was isolated on culture in 7 to 50% of the organs of the indigenous birds. During the same period four (lung, trachea/oropharynx, liver and spleen) organs on FISH test and one (trachea/oropharynx) on culture were throughout positive for P. multocida. The large intestine/cloaca and pruning gland showed P. multocida FISH signals at various times but were negative for the bacterium on culture. Both tests were positive for P. multocida immediately after inoculation. FISH signals were found in a decreasing manner in the lung, trachea/oropharynx, liver, spleen, coecal tonsils, large intestine/cloaca, and pruning gland. On culture, the bacteria were found in a decreasing manner in the trachea/oropharynx, lung, spleen, liver and coecal tonsils. Most cultured isolates were made between 1-24 hours, few and intermittent ones thereafter and none at all after the 10th day post infection. These results show that FISH test is more sensitive than the culture method for detection of P. multocida in tissues of infected birds.

Materials and Methods
Experimental birds: A total of forty-eight indigenous birds (24 twelve week-old chickens and 24 eight week-old ducks) were used in this study. All were reared in isolation, screened severally through bacteria culture of swabs taken from their cloaca and oropharynx and found negative for P. multocida before experimentation.

Bacteria and infection of the birds: Pasteurella multocida strain NCTC 103227 kindly provided by Prof. Magne Bisgaard and maintained on Dorset egg agar, in our laboratory, was used in this study. It was spread onto blood agar (5% citrated calf blood), incubated aerobically at 37°C, for 24 hours to check for purity prior to preparation of the inoculum as described by Petersen et al. (2001). Each bird was inoculated intratracheally with 0.5 ml of brain heart infusion (BHI) broth culture containing 1.2-1.9 x 10⁸ P. multocida organisms. Biosafety measures were maintained during the entire period of the study.

Sample collection from the experimental birds
A total of 4 birds (2 chickens and 2 ducks) were killed through cervical dislocation at hours 0 (1-5 minutes), 1, 3, 6, 12, 24 and days 2, 3, 5, 7, 10, and 14 post infection. The dead birds were opened aseptically and lung, trachea/oropharynx, liver, spleen, coecal tonsils, large intestine/cloaca and pruning gland were sampled from each of the 48 experimental birds for bacterial and histological-FISH examination.

but non-culturable cells (Krause et al., 1987). Serological methods are complicated by non-typable strains and cross-reaction during sero-typing (Rimler et al., 1998). The FISH test depends on the number of copies of ribosomal ribonucleic acid in the cell (Amman et al., 1995), and hence on the physiological activity of the microorganisms prior to fixation of the samples (Delong et al., 1989). In this study the sensitivity of two techniques that are specific for P. multocida were evaluated in their ability to detect the bacteria in organs of experimentally infected birds.