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# Salmonella live attenuated vaccine preparation.

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- 1 A Salmonella cocktail (mixture of five Salmonella strains: S. Montevideo, S. Yeerongpilly, S. Augustenborg, S. Kentucky and S. Typhimurium) is incubated at 37°C in peptone broth with combinations of natural extracts.
- 2 Preparation includes clean, sun dried onion and garlic cloves.
- 3 After a week of drying, onion and the cloves of garlic is separately grounded using electric blender,
- 4 Dilute the plant preparations in 1L distilled water.
- 5 Each extract is used individually and in combined concentration.

- 6 Five ml of the diluted extract (15 mg/ml) is incorporated in differential and selective Salmonella plating media.
- 7 A bacteriogram is carried out to check for bacteria attenuation.
- 8 The immunogen contains 5 serovars of Salmonella ( $10^8$  CFU of vaccine strain) per chicken in 1 ml of incomplete Freund's adjuvant.
- 9 It is given intramuscularly at chicken breast
- 10 The immunogen (5 serovars) without incomplete Freund's adjuvant (IFA) is given orally ( $10^8$  CFU per chicken).
- 11 All Salmonella isolates were investigated for their antibiotic resistance with the disc diffusion test using the following discs (Difco):
- 12 gentamicin (10 µg), kanamycin (30 µg), ampicillin (10 µg), amikacin (30 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), chloramphenicol (30 µg), cefazolin (30 µg), cephalothin (30 µg), cefepime (30 µg), cefotaxime (30 µg), streptomycin (10 µg), ceftiofur (30 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), norfloxacin (10 µg), tetracycline (30 µg) and imipenem (10 µg).
- 13 The five Salmonella serovars were fully attenuated and inoculated in appropriate media and store at -20 degree Celsius.