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BACTERIOLOGICAL FINDINGS REGARDING THE HYGIENIC SAFETY OF POULTRY LITTER INTENDED AS AN INGREDIENT OF FEEDS FOR RUMINANTS

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ABSTRACT

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An investigation of poultry litter intended for use in farm feeds showed that 0,37 %, 0,49 %, 0,25 % and 12,3 % of the 813 samples tested were contaminated with *Clostridium* spp., haemolytic *Escherichia coli*, *Staphylococcus aureus* and 21 different species of *Salmonella*. The findings clearly underline the hygienically dangerous nature of crude poultry litter. The practical implications of the results are briefly discussed, particularly in view of current regulations.

INTRODUCTION

Depending on its level of uric acid and other constituents, poultry litter may be used in feeds for ruminants as a valuable source of nitrogen, provided it is also hygienically safe. The significance to animal and human health of poultry litter as an ingredient of ruminant feeds has been the subject of several investigations abroad (Messer, Lovett, Murthy, Wehby, Schafer & Read, 1971; Strauch, 1983). The data indicate that the health hazards most obviously associated with feeding poultry litter to livestock are those caused by residues of chemicals (e.g. non-antibiotic and antibiotic remedies) from medicated feeds, pesticides, certain pathogenic microbial toxins (e.g. from *Clostridium botulinum*) and bacteria (e.g. *Salmonella* spp.), all possibly present in the litter.

Owing to its importance as supplementary feed for ruminants and as a potential hazard to health (Centre of Disease Control, 1979), poultry litter sold as animal feed in South Africa is subject to the official control of the Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act, 1947 (Act 36 of 1947).

Regulation R. 1359 and other related regulations (*Government Gazette*, 1980) are explicit in their requirements for various physical and chemical properties of poultry manure [section 12 (2)]. However, the microbiological requirements [section 10 (4) (b)] seem to be inconsistent and vague for the following reasons:

1. They stipulate that a registered farm feed, such as poultry manure, must be sterilized.
2. They also point out, however, that by means of such a sterilization infection or contamination with harmful micro-organisms or substances has to be reduced to levels not injurious, dangerous or detrimental to animals fed with such a product.
3. They do not specifically mention any standard methods for the microbiological screening of registered farm feeds nor any other techniques, parameters and criteria for determining their injurious, dangerous or detrimental nature to animal health and production.

Poultry manure is a potentially hazardous ingredient in feed for ruminants and its control in terms of Act 36 of 1947 is an important factor in preventive medicine. Its importance as a source of infection and the necessity for its control have been sharply emphasized in recent months when manure has been proposed for relieving the shortage of farm feeds caused by the severe drought. As an emergency measure the Registrar of Act 36 of 1947 was appealed to, to permit the sale of non-sterilized poultry litter as farm feed. It was therefor deemed necessary to launch an investigation on the safety of non-sterilized poultry litter from the hygienic point of view.

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During studies on the poultry litter and from investigations on various commercial mixed feeds (Ogonowski, unpublished data, 1983), it became evident that, although extensive determinations of pathogenic bacteria (e.g. *Salmonella* spp.) have been performed on foods, fewer determinations have been made on animal feeds, and only very limited information is available on practical techniques for collecting samples of poultry manure and screening them for pathogenic micro-organisms. The aim of this investigation was to augment the information on the bacterial content of poultry manure and to provide some relatively detailed descriptions of some of the techniques used during the investigation.

MATERIALS AND METHODS

Poultry litter examined

The 813 samples of poultry litter examined were collected at 87 poultry farms distributed throughout the RSA (Fig. 1).

Collection of samples

Different types of fresh/old and dry/moist composite litter and almost pure manure were collected under the varying conditions of broiler, egg and breeding stock production. Where possible, samples were taken from masses of litter freshly removed during routine cleaning operations from the production units. In a few exceptional cases, samples of litter were collected in poultry houses at sites chosen at random. Samples of approximately 200–500 g each were taken at various sites and depths from each batch of litter.

The samples were taken by inspectors of the Registrar Act 36/1947, who have great practical experience in the sampling of bulk consignments of different products. They are specially trained to collect samples with hygienic precautions, both to protect the consignments from infection and prevent them from contaminating other products and samples intended for microbiological investigations.

Samples were collected in new 255 × 380 mm bags of transparent plastic. Preparatory to the sampling, an approximately 100 mm-wide strip of the orifice of the bag was turned over the fingers of one hand, without touching the internal surface of the bag. With the hand palm upwards, the straightened fingers with their glove-like plastic protection were then pushed in a scooping forward motion through the litter until an adequate amount of it was collected in the rest of the bag lying on the palm. The bag was then closed by tightly twisting its empty portion and placing it in a new 800 mℓ tin. This, in turn, was immediately closed and the lid completely sealed with a portable tool. Each sample container was then properly identified by means of a certificate recording detailed information, a corresponding sticker label

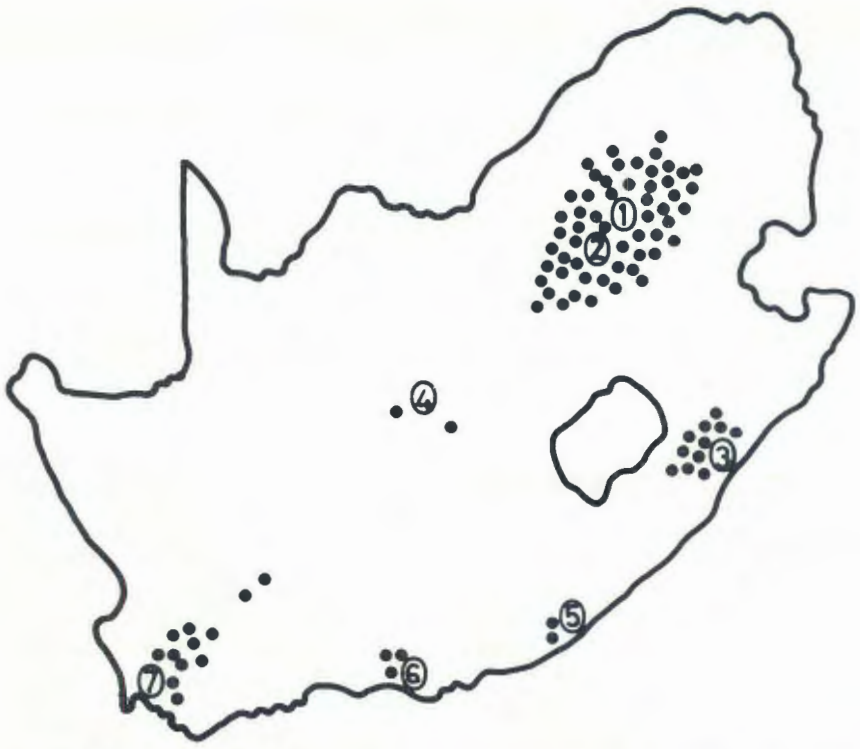

FIG. 1 Sketched map of the RSA. Nos. 1, 2, 3, 4, 5, 6 and 7 = Pretoria, Johannesburg, Durban, Bloemfontein and Kimberley, Port Elizabeth, East London and Cape Town respectively. Each dot represents one poultry farm investigated.

affixed to the tin and a corresponding sample number recorded on the certificate, the label and the lid of the tin proper.

The sealed samples were then transported together with their certificates to the Veterinary Research Institute, Onderstepoort, where they were registered and transferred to the Section of Food Hygiene for further processing at the laboratory.

Laboratory examination

The samples were examined mainly for the presence of certain pathogenic bacteria. Additional examinations on several of the samples were performed for investigating standard plate counts of general bacterial contaminants.

1. *Isolation of* Salmonella *spp.*

Salmonella spp. were determined by means of a method, the individual steps of which are described below.

(1) An aliquot of 20 g of each sample was placed in a 250 mℓ sterile conical flask to which was added 200 mℓ of sterile peptone water containing Tween 80 at a final concentration of 0,1 %. The suspension was mixed and incubated for 20–24h at ± 37 °C.

(2) 1 mℓ of supernatant fluid of the incubated suspension (1) was used for inoculating 20 mℓ of SBM broth* (i.e. enrichment medium containing selenite, brilliant green and manitol) and incubated for 20–24h at ±37,5 °C.

(3) After incubation, 1 loopful (0,01 mℓ) of SBM bouillon was spread on

(a) SS agar* (i.e. *Sheigella* and *Salmonella* agar) and

(b) bismuth-sulphite agar.*

Both plates were then incubated for 20–24 h at ± 37,5 °C.

(4) After incubation of 3a and 3b, stab cultures in triple sugar iron agar slants (TSI*) were prepared, using colourless, translucent single colonies picked from SS plates (3a) and suspect *Salmonella* colonies.

Both the TSI stab cultures were incubated again for 20–24 h at ± 37,5 °C.

(5) The TSI slant cultures were assessed for growth of *Salmonella* spp.

(6) A single suspect colony was removed from either 3a and 3b or from 4 for further testing by means of the API-20E series.†

(7) All cultures API positive for *Salmonella* spp. were serotyped by the Section of Bacteriology.

(8) A sample was identified as positive for *Salmonella* where culturing, API testing and serotyping were clearly positive both in terms of growth and of the *Salmonella* spp. isolated.

(9) If any of the cultures, API testing or serotyping, produced doubtful results, another sample of 20 g of originally registered sample was re-examined.

(10) A sample was *Salmonella* negative when culturing, API testing and serotyping gave clearly negative results.

2. *Biological test for* Bacillus anthracis *and* Clostridium *spp.*

(1) 20 g of sample was placed in a sterile specimen jar filled with sterile saline, agitated, and put into a water-bath for 60 min at 60 °C.

(2) After cooling, 1 mℓ each of supernatant fluid was administered intramuscularly to 2 adult healthy guinea-pigs.

(3) The guinea-pigs were kept under close observation in a well-ventilated room and regularly supplied for 12 days with food and water.

* Merck Chemicals, South Africa

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† API Systems, France

(4) If no deaths occurred during the 12 days, the corresponding guinea-pigs and sample were considered free from *B. anthracis* and *Clostridium* spp.

(5) Dead guinea-pigs were subjected to a post-mortem and bacteriological investigation for *B. anthracis* and *Clostridium* spp. For the latter, pieces of spleen, liver and heart were used to prepare in triplicate, impression smears on blood tryptose agar plates and then put into a test-tube containing cooked mincemeat broth. The plates and the tubes were incubated aerobically, anaerobically and in a candle jar for 24 h at 37,5 °C. After incubation a loopful of an inoculated meat broth was plated on the blood tryptose agar plates and incubated again for 24 h at 37,5 °C. Haemolytic colonies from blood tryptose agar plates were picked and replated on fresh blood tryptose agar plates to obtain pure cultures. These were sent to the Section of Bacteriology for tentative identification.

(6) If dead guinea-pigs were positive for *B. anthracis* or *Clostridium* spp., another 20 g of the original sample was investigated on 2 new guinea-pigs, using procedures 1–5.

3. *Methods for isolating other pathogenic bacteria*

(1) *S. aures* was isolated from aerobic blood tryptose agar plates inoculated during the isolation procedure for *B. anthracis* and identified by the Section of Bacteriology.

(2) *E. coli:* 1 g of the sample was transferred to a McCartney bottle with 15 mℓ of MacConkey broth and incubated in a water-bath for 48 h at 44 °C. A loopful of incubated MacConkey broth was spread on blood tryptose agar and MacConkey plates. After 24 h incubation at 37,5 °C, the suspect colonies were picked up, inoculated on blood tryptose agar plates until cultured pure and then sent for tentative identification to the Section of Bacteriology.

(3) *Standard plate count:* 1 g of each sample was suspended in 9 mℓ of phosphate buffer in a 20 mℓ McCartney bottle. After agitating the suspension, 3 tenfold dilutions were prepared from it in phosphate buffer. One drop (0,05 mℓ) from each dilution was plated on TGY-agar* and Wickerham† agar and incubated at 37 °C for 24 h and at 28 °C for 48 h respectively. Colony forming units on Wickerham plates were counted visually, and those on TGY plates with a Biomatic 16900 colony counter‡. The counts per mℓ of phosphate buffer were then calculated.

RESULTS

Prevalence of pathogenic bacteria other than Salmonella *spp.*

Clostridium spp., pathogenic *E. coli* and *S. aureus* were isolated respectively from 0,37; 0,49 and 0,25 % of the 813 samples investigated.

Further identification of the 3 isolates of *Clostridium* spp. showed 2 *C. septicum* 1 each from 2 different farms in Transvaal and 1 *C. perfringens* from a farm in Natal.

The 4 isolates of pathogenic *E. coli* each originated from 3 different Transvaal units and from 1 farm in the Cape Province.

The 2 isolates of *S. aureus* both originated from a Transvaal farm.

Prevalence of Salmonella *spp.*

Salmonella spp. were isolated 103 times from 100 (i.e. 12,3 %) of the 813 samples examined. The 103 isolates of *Salmonella* spp. were obtained from 31 of the 87 farms investigated (20 in the Transvaal, 6 in Natal, 4 in Cape and 1 in Orange Free State). Of the 55 Transvaal, 19 Cape, 11 Natal and 2 Orange Free State farms investigated, those infected with *Salmonella* spp. were 36,4; 21,1; 54,6 and 50 % respectively of the corresponding farms examined per province.

Further identification of the 103 isolates of *Salmonella* spp. showed that the samples were contaminated with a variety of species (Table 1).

TABLE 1 *Salmonella* spp. isolated from poultry litter and the corresponding frequency of isolation

Salmonella spp.	Number of isolates
S. senftenburg	29
S. chester	14
S. remo	10
S. nitra	6
S. naware	5
S. makumira	4
S. tafo	4
S. heidelberg	2
S. typhimurium	2
S. jaja	2
S. bradford	2
S. hessarek	2
S. nyeko	2
S. tilburg	2
S. orientalis	1
S. selandia	1
S. messina	1
S. jubliana	1
S. fulica	1
Salmonella II	1
Salmonella (rough)	11

Additional *ad hoc* investigations on standard plate counts of 19 samples and the moisture content (%) of 22 samples selected at random indicated mean values ± standard deviations of $1544{,}26 \times 10^6 \pm 1374{,}26 \times 10^6$ colony forming units per gram of sample, and 18,3 ± 8,24 % moisture content. Only 1 of the 21 samples tested showed a moisture content (9,5 %) within current requirements.

DISCUSSION

The isolation from poultry litter of a variety of pathogenic bacteria and a wide range of *Salmonella* spp. is consistent with the results of other workers (Messer *et al.*, 1971; Strauch, 1983).

From the point of view of veterinary hygiene the finding thus clearly confirms the potentially dangerous nature of crude poultry manure. It further implies that under conditions permitting the selling as farm feed of unsterilized poultry litter, a considerable number of poultry production units may be considered potential sources for the general spreading of especially *Salmonella* spp.

From the point of view of preventive veterinary medicine the results clearly emphasize the importance of appropriate field and laboratory investigation, the necessity of follow-up measures at the farm and the urgent need for greater vigilance regarding the countrywide dissemination of *Salmonella* not only by poultry litter as such but probably also by poultry farms and abattoirs in general.

* Oxoid
† Wickerham (1951, cited by Giesecke, Nel & Van den Heever, 1968)
‡ A/SN Foss Electronic, Denmark

Our findings on contamination with pathogens, moisture content (%) and standard plate counts underline the conclusion that unless crude poultry litter is sterilized it should not be registered as farm feed, however great the shortage of feedstuffs. The necessity for sterilizing poultry litter is nothing new (Messer *et al.*, 1971); it has recently been re-emphasized elsewhere (Strauch, 1983), and is now confirmed by this investigation. It is surprising, therefore, that the current regulations for the sterilization of poultry litter and other farm feeds intended for registration are not as specific as the situation demands. This is further aggravated by the fact that poultry litter, though frequently heavily contaminated with pathogens, or hygienically unacceptable for other reasons, and therefore clearly unregistrable as a farm feed, may still apparently be distributed non-sterilized for other purposes (e.g. fertilizer), though it constitutes a danger to animal and public health. The present investigation suggests that amendments aimed at the elimination of such inconsistencies are necessary.

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