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014 - Salmonella IV

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Isolation and identification of salmonella

We isolate and identify salmonella for two main reasons. Firstly, we use the isolation of the salmonella organism from the organs of a bird that we suspect of dying of salmonellosis for the purpose of confirming our diagnosis. Secondly, we want to isolate salmonella from a variety of materials such as faeces, environmental dust, eggs and feed to confirm whether or not the salmonella is present in the material tested.

In the latter situation we will need to test a predetermined number of samples so that we can give a degree of confidence to our result(s). This is especially important in large flocks with a low level of infection. Typically, we want to test with a 95% confidence limit but, in some situations, this might need to be the 99% level.

Isolation from organs

Highly invasive salmonella serotypes, such as Salmonella enteritidis, can be disseminated to many tissues including ovary, spleen, liver, oviduct, testes, yolk sac, heart, heart blood, kidney, gall bladder, joint fluid/pus and the eyes and all of these can be cultured as part of a post mortem examination. As salmonella often colonise the intestinal tract this and its contents should also be cultured. However, isolation from the intestinal contents per se is not diagnostic of salmonellosis – for a diagnosis isolation should occur from the internal organs.

Isolation from organs can be done by direct culture on to blood and MacConkey agar and salmonella selective agars such as XLD or brilliant green agar. In such cases suspect colonies should be subjected to further chemical and serological tests to confirm that they are, in fact, salmonella, before they are serogrouped and serotyped.

Isolation from other samples

When testing other samples, such as feed or litter, it would be reasonable to expect any salmonella bacteria to be present as a very small minority and this provides some challenges for the bacteriologist. These are overcome by following a protocol that includes non-selective pre-enrichment, selective enrichment and, finally, culturing on to a selective agar.

The objective of non-selective pre-enrichment using an enrichment broth, such as buffered peptone water, is to encourage the growth of the few salmonella present and where cells are injured to hopefully resuscitate them. After incubation an aliquot of this broth is transferred to a selective enrichment broth.

In selective enrichment the broth is designed to favour the growth and multiplication of salmonella bacteria, while inhibiting the growth and multiplication of other bacteria, so that when this broth is ultimately streaked on to a selective agar the ratio of salmonella:other bacteria has swung greatly in favour of the former.

On selective agars salmonella bacteria produce distinctive colonies and these are then selected for further identification and confirmatory work.

Confirmation of salmonella

Suspect colonies from the selective agar plate can be subjected to chemical tests to confirm that they are salmonella. In addition, pure growths of the isolate can be subjected to agglutination tests using polyvalent salmonella antisera. These are often referred to as poly O and poly H – the former containing polyvalent antisera to the O or somatic antigens and the latter containing polyvalent antisera to the H or flagellar antigens.

Rapid methods

Traditional salmonella culture often takes several days, although negative results take a shorter time so faster methods have been developed.

There are cost and sensitivity issues associated with rapid methods and many countries do not recognise them for testing taking place as part of a national control programme or for international trade in poultry products purposes.