The British Veterinary Poultry Association has recently agreed guidelines on the use of antimicrobials in poultry (Thorp and McMullin 1998), including an addendum specifically directed at fluoroquinolone use, and this is to be welcomed. It is important that these guidelines are strictly followed so that the activity of lifesaving antibiotics can be preserved for as long as possible. In addition, it is advisable that turkey and other livestock produces should take steps to upgrade salmonella control measures, so that *S typhimurium* DT104 is not allowed to persist on farms or in hatcheries, or to spread from cattle herds on the same farms as poultry. It is also desirable that any trends which are identified by national veterinary surveillance schemes, and which may represent a current or potential threat to human health, are reported to specialist livestock organisations so that appropriate action can be taken.

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Prevalence of antibodies to bovine paratuberculosis detected by a LAM-ELISA in Costa Rica

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Mycobacterium paratuberculosis generally causes a subclinical infection (Brugere-Picoux 1987) in which about 10 per cent of infected animals develop the clinical form (Johne's disease) after a prolonged incubation period. Clinically, a chronic debilitating enteritis is observed and domestic ruminants, such as cattle, sheep and goats, and some wild ruminants are affected. The agent is enzootic throughout the world and causes significant economic losses in dairy cattle (Cocito and others 1994).

M paratuberculosis is a slow growing, fastidious organism and direct diagnosis with primary cultures takes between nine and 16 weeks of incubation before colonies can be observed (Chiodini and others 1984). However, the organism is shed intermittently from the intestine and substances used to control normal flora could inhibit the growth of some strains of *M paratuberculosis* (Ridge 1993). Biochemical tests used with other mycobacteria have not proved to be reliable in the iden-

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tification of *M paratuberculosis* (Chiodini and others 1984). Cutaneous testing with antigens obtained from the organism has been used as a diagnostic procedure in several countries including Costa Rica (Evans and Vargas 1991). *M paratuberculosis* extracts are often replaced by *Mycobacterium avium* preparations as the latter is more easily available. The use, however, of heterologous antigens decreases the specificity of the test, making comparative cutaneous testing with both avian and bovine tuberculins necessary. Large numbers of false positive and false negative reactors also occur (Gilot and Cocito 1993).

In Costa Rica the diagnosis of Johne's disease has been based on clinical, histopathological and bacteriological evidence and cutaneous testing; however, the prevalence of the disease at the national level is unknown. Since serum ELISA is a well accepted method of testing for antibody response to *M paratuberculosis* (Collins and others 1991, Sockett and others 1992), a bovine serum bank collected in 1990 and a lipoarabinomannan antigen ELISA (LAM-ELISA) which included a preabsorption step with *Mycobacterium phlei* (Sudgen and others 1987, 1989) was used.

Sera were obtained from a bovine serum bank composed of 4352 samples collected in 1990 by the Ministry of Agriculture of Costa Rica for a national brucellosis survey. From this serum bank 654 samples were selected proportionally to the cattle population of each region of Costa Rica (confidence 99·5 per cent, error 5 per cent, $P<0·05$). Seven positive sera and seven negative sera were used as controls.

The lipoarabinomannan (LAM) antigen used was purified from *M paratuberculosis* strain V. The LAM-ELISA was conducted according to the test protocol submitted by Dr J. R. Duncan of the Animal Diseases Research Institute, Ottawa, Canada, with some modifications. Control and test sera were diluted at a ratio of 1:200 in 0·01M PBS, and 8 µg of *M phlei* antigens were added to each serum. The sera were absorbed overnight at 4°C, and centrifuged for five minutes at 12,000 rpm in an Eppendorf centrifuge before use in the LAM-ELISA. Polysorb microtitre plates (Nunc) were coated with 200 µl LAM antigen (0·2 µg antigen per well) diluted in 0·05M carbonate buffer, pH 9·6, and incubated overnight in a humid chamber at room temperature. The microtitre plates were washed four times with washing buffer (0·02M PBS, 0·1 per cent Tween-20). Positive control serum, negative control serum and test sera were added to the plates and incubated for two hours at 37°C. After washing the plates, the peroxidase conjugate, a mouse monoclonal antibody against bovine IgG_1(M23) was diluted at a ratio of 1:20,000 in 0·01M PBS, 0·1 per cent Tween-20, added to each well and the plate was incubated for two hours at 37°C. The plates were washed four times and substrate (ABTS) was added. The optical density (OD) was measured at 405 nm in a Multiscan Titertek. The reaction was stopped when the positive control serum reached an OD of 0·500. The optical densities of the test sera were expressed as a positive percentage (PP) with respect to the OD of the positive control of each plate (OD of positive control sera=100 per cent PP).

All control negative sera showed optical densities below 0·072, whereas the positive control sera yielded optical densities greater than 0·270. The negative cut-off value was set as the arithmetic mean of all negative control sera plus three standard deviations. Under this criteria, test sera with an OD greater than 0·200 or a PP greater than 40 per cent were considered positive in this investigation.

From 654 bovine sera analysed, 78 sera (11·9 per cent) reacted positive under the LAM-ELISA with *M phlei* absorption. From these 78 positive reacting sera, 38 (48·7 per cent) corresponded to animals aged between four and six years. A total of 68 (18·7 per cent) herds out of 364 tested had positive reactors to *M paratuberculosis*. The positive herds were distributed throughout the country (Table 1).

The present study is the first attempt at determining the national seroprevalence of bovine paraturberculosis in Costa Rica, and to the authors' knowledge, is the first report in Central America. Exposure to the agent was demonstrated in 11·9 per cent of the tested animals and in 18·7 per cent of the herds nationwide. The determined prevalence is similar to that reported in a study in Canada (Chiodini and Van Kruiningen 1986), however, the percentage of herds exposed to the agent might increase with greater sample size, since very few animals were tested in some herds.

TABLE 1: Detection of antibodies to *M paratuberculosis* from cattle in different geographical regions of Costa Rica using LAM-ELISA and *M phlei* absorption

Geographical region	Bovines Reacted/tested (%)	Herds Reacted/tested (%)
Pacifico Central	11/61 (18·0)	9/26 (34·6)
Central Oriental	4/28 (14·3)	4/15 (26·7)
Central	8/59 (13·9)	7/31 (22·5)
Central Occidental	2/30 (6·7)	2/20 (10·0)
Pacifico Seco	26/209 (12·4)	22/121 (18·2)
Pacifico Sur	6/55 (10·9)	5/41 (12·1)
Huetar Atlántica	10/88 (11·4)	8/44 (18·1)
Huetar Norte	11/124 (8·9)	11/66 (16·7)
Total	78/654 (11·9)	68/364 (18·7)

The presence of the agent throughout the country was confirmed, and prevalence determined in the different regions of Costa Rica. It would be desirable to confirm these results against a larger sample size, including ecological, age and management information, which may shed some light regarding the risk factors for the disease in this geographical area.

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