Research in Veterinary Science 95 (2013) 45–48

Contents lists available at SciVerse ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Wild birds carry similar *Salmonella enterica* serovar Typhimurium strains to those found in domestic animals and livestock

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ARTICLE INFO

Article history:
Received 18 September 2012
Accepted 7 February 2013

Keywords:
Salmonella Typhimurium
Wild birds
Domesticated animals
Livestock
MLVA
PFGE

ABSTRACT

The objective of this study was to investigate the hypothesis that some sporadic *Salmonella* infections in domesticated animals may be associated with *Salmonella* infections originating from garden birds. Phage type and antimicrobial resistance details of isolates of *S.* Typhimurium obtained from wild birds were comparable with those from *S.* Typhimurium infections from domesticated animals or livestock between 2002 and 2010. A small panel of *S.* Typhimurium isolates ($n = 37$) were characterised by multilocus variable number of tandem repeats analysis (MLVA), pulsed field gel electrophoresis (PFGE) and phage type. The MLVA-PFGE data clustered the strains according to phage type (DT40 or DT56). Within each group there were strains from wild birds and domesticated animals or livestock with MLVA profiles having up to 100% similarity. The results from this study therefore lend support to the hypothesis that *Salmonella* infections in domesticated animals could be caused by infections carried by wild birds.

The potential health risks to humans and livestock from *Salmonella* infections in wild birds has long been recognised (Wilson and MacDonald, 1967). Recent evidence suggests that salmonellosis is the most frequent cause of death from infectious disease in garden birds, with house sparrows (*Passer domesticus*) and greenfinches (*Carduelis chloris*) being particularly susceptible (Lawson et al., 2010). The predominant strains of *S.* Typhimurium responsible are within definitive phage types (DT) DT40 and DT56 (Pennycott et al., 2006). There have been a number of reports of sporadic outbreaks of salmonellosis in domestic animals (particularly cats) and livestock thought to be associated with *Salmonella* infections in wild birds (Alley et al., 2002; Refsum et al., 2002; Pennycott et al., 2006; Philbey et al., 2008; Taylor and Philbey, 2010). Human cases have also been reported (Kapperud et al., 1998; Pennycott et al., 2006). The objective of the present study was therefore to investigate the hypothesis that some sporadic *Salmonella* infections in domesticated animals or livestock may be associated with *Salmonella* infections originating from garden birds.

AHVLA surveillance records of the yearly frequency of isolates of *S.* Typhimurium from wild birds (between 2002 and 2010) were compared to AHVLA records of the frequency of incidents of *Salmonella* infections for domesticated animals and livestock over the same time period. A small panel of *S.* Typhimurium isolates ($n = 37$) was selected from those available in the AHVLA bacteriology archive (selected for DT40 and DT56 phage types from wild birds, livestock or domesticated animals between 2002 and 2010, and selected irrespective of antimicrobial resistance/susceptibility pattern) to achieve a set of representative isolates with diverse dates of isolation and source species, for analysis by MLVA and PFGE. The panel consisted of isolates obtained from wild birds including: greenfinch ($n = 8$), siskin ($n = 5$), house sparrow ($n = 3$), bullfinch ($n = 2$), chaffinch ($n = 1$), and goldfinch ($n = 1$), and other isolates from domestic animals and livestock including: cattle ($n = 3$), cats ($n = 4$), horses ($n = 3$), dogs ($n = 3$), chickens ($n = 3$) and a pig farm ($n = 1$). The majority of these isolates (26/37) were fully susceptible to all 16 antimicrobial drugs (amikacin, ampicillin, amoxicillin/clavulanic acid, apramycin, chloramphenicol, ceftazidime, ciprofloxacin, furazolidine, gentamicin, cefotaxime, nalidixic acid, neomycin, streptomycin, sulphonamide compounds, sulphamethoxazole/trimethoprim and tetracycline) that are used in routine antimicrobial susceptibility testing of *Salmonella* (Andrews, 2009; Veterinary Laboratories Agency, 2010). The single exception was one DT56 isolate from a house sparrow (L01727-02), that was resistant to sulphonamide compounds only, and yet still possessed 100% similarity in terms of PFGE and MLVA profiles with 4 other fully sensitive isolates (Fig. 1). The wild birds were found either dead or wasting and the majority of these were diagnosed with clinical salmonellosis. The main clinical sign in the cats, dogs and cattle was diarrhoea.

Surveillance data from between 2002 and 2010 suggested that the occurrence of *S.* Typhimurium infections in wild birds, as judged by the number of laboratory reports, (Fig. 1a) may have

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http://dx.doi.org/10.1016/j.rvsc.2013.02.008

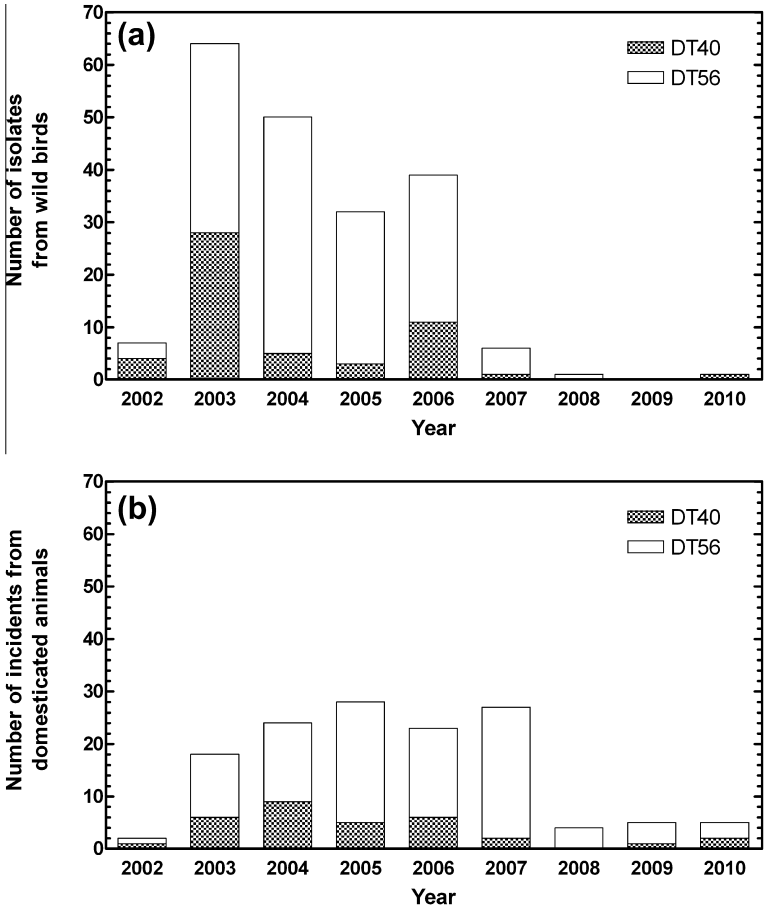

Fig. 1. Frequency of *Salmonella enterica* serovar Typhimurium (DT40, and DT56) detection recorded by AHVLA surveillance from 2002 to 2010. (a) number of isolates from wild birds: greenfinch ($n = 93$), siskin ($n = 20$), house sparrow ($n = 30$), bullfinch ($n = 11$), chaffinch ($n = 5$), goldfinch ($n = 2$), herring gull ($n = 2$), tree sparrow ($n = 2$), tawny owl ($n = 1$) and unidentified ($n = 33$). (b) Number of Incidents in pets and domesticated animals: horses ($n = 53$), cats ($n = 20$), cattle ($n = 17$), dogs ($n = 11$), chickens ($n = 9$), alpaca ($n = 3$), pigs ($n = 3$), sheep ($n = 3$), turkeys ($n = 2$), cockatoo ($n = 1$), mixed avian species ($n = 1$), parrot ($n = 1$), pigeon ($n = 1$) and unknown ($n = 1$).

increased significantly between 2002 and 2003, due to unknown factors. The number of reported cases was maximal in 2003 with approximately equal representation of the phage types studied (DT40 and DT56) in that year. The yearly number of reports of wild bird isolates declined steadily over the following 3 years, but with an apparent increase in the proportion of DT56 strain types during that period. The data for wild bird submissions are likely to be underestimated post 2006 due to the establishment of the Garden Bird Health initiative and the subsequent submission of birds to that scheme in preference to AHVLA regional laboratories. Incidents involving domesticated animals also showed a marked increase between 2002 and 2003 (Fig. 1b), although the overall yearly profile was flatter (the frequency of incidents in domesticated animals was approximately constant between 2004 and 2007) compared with the profile for wild birds. There was a marked decline in the number of incidents in domesticated animals post 2007 and again the majority of these latter incidents involved the DT56 strain. The correlation coefficients for comparisons of the annual frequency of wild bird isolates and domestic/livestock incidents with respect to DT40 or DT56 were calculated by first transforming the data to $\log_{10}$ (count + 1). The correlation coefficients for these comparisons for both phage types were greater than 0.7 and were statistically significant ($p < 0.05$). Although such comparisons do not imply a causal relationship, they do confirm an association in the trends over time. The surveillance data is therefore consistent with the hypothesis that the prevalence of *Salmonella* infections in domesticated animals may be influenced by the prevalence of infections in wild birds.

MLVA analysis was conducted on isolates by using a five loci scheme (Larsson et al., 2009), that assigns allele numbers based on actual number of repeat units in each locus. Briefly, DNA templates were prepared and amplification was achieved by using a multiplex PCR kit (Qiagen Ltd, West Sussex, UK) with standard unmodified oligonucleotides (MWG-Biotech AG, Ebersberg, Germany) as reverse primers (STTR3-R, TGA CGC CGT TGC TGA AGG TAA TAA; STTR5-R, GGT CAG GCC GAA TAG CAG GAT; STTR6-R, CTG GTG GGG AGA ATG ACT GG; STTR9-R, CAT TTT CCA CAG CGG CAG TTT TTC and STTR10R, GAA GGG GCC GGG CAG AGA CAG C) and florescence-labelled forward primers (PET-STTR3-F, CCC CCT AAG CCC GAT AAT GG; VIC-STTR5-F, ATG GCG AGG CGA GCA GCA GT; NED-STTR6-F, TCG GGC ATG CGT TGA AA; FAM-STTR9-F, AGA GGC GCT GCG ATT GAC GAT A and FAM-STTR10-F, CGG GCG CGG CTG GAG TAT TTG).

PFGE analysis was performed by following the standard PulseNet$_{USA}$ protocol (Ribot et al., 2006) after macro-restriction of bacterial DNA with *Xba*1 endonuclease. This produces a characteristic PFGE profile for each isolate consisting of different DNA fragment sizes. The PFGE profiles produced by each isolate were compared alongside the respective MLVA profiles and the combination of MLVA and PFGE data were analysed with BioNumerics software V. 3.00 (Applied Maths, Kortrijk, Belgium). The dendrogram of MLVA–PFGE combined profiles of the isolates (Fig. 2) could be separated into three groups (A, B and C) based on >60% similarity. Groups A and B corresponded largely to the two phage types, DT56 and DT40 respectively. There was a higher proportion of isolates from wild birds (78%) in group A (DT56) than within group B