

This is a repository copy of '*Disperse abroad in the land*':the role of wildlife in the dissemination of antimicrobial resistance.

White Rose Research Online URL for this paper:

<https://eprints.whiterose.ac.uk/104508/>

Version: Accepted Version

Article:

Arnold, Kathryn E orcid.org/0000-0002-6485-6065, Williams, Nicola J and Bennett, Malcolm (2016) '*Disperse abroad in the land*':the role of wildlife in the dissemination of antimicrobial resistance. *Biology letters*. ISSN 1744-957X

<https://doi.org/10.1098/rsbl.2016.0137>

Reuse

Items deposited in White Rose Research Online are protected by copyright, with all rights reserved unless indicated otherwise. They may be downloaded and/or printed for private study, or other acts as permitted by national copyright laws. The publisher or other rights holders may allow further reproduction and re-use of the full text version. This is indicated by the licence information on the White Rose Research Online record for the item.

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

1 **Review: ‘Disperse abroad in the land’: The**
2 **role of wildlife in the dissemination of**
3 **antimicrobial resistance**

4 Kathryn E. Arnold¹, Nicola J. Williams² and Malcolm Bennett³

5 ¹ Environment Department, Faculty of Sciences, University of York, Heslington, York
6 YO10 5NG, UK

7 ² Department of Epidemiology and Population Health, Institute of Infection and
8 Global Health, Leahurst Campus, University of Liverpool, Neston, CH64 7TE, UK

9 ³ School of Veterinary Medicine and Science, The University of Nottingham, Sutton
10 Bonington Campus, Sutton Bonington, Leicestershire, LE12 5RD, UK

11

12 Corresponding Author: Kathryn Arnold, Environment Department, Faculty of
13 Sciences, University of York, Heslington, York YO10 5NG, UK

14 Email: Kathryn.Arnold@york.ac.uk

15 Phone: 01904 322997

16

17 Running head: AMR dispersal by wildlife

18 Key words: antibiotic resistance, migration, disease transmission, animal dispersal,
19 resistome, sewage treatment

20 Word count including references: 5551

21

1 **Abstract**

2 Antimicrobial resistance (AMR) has been detected in the microbiota of many wildlife
3 species, including long distance migrants. Inadequately treated wastes from humans
4 and livestock dosed with antimicrobial drugs are often assumed to be the main
5 sources of AMR to wildlife. While wildlife populations closely associated with human
6 populations are more likely to harbour clinically important AMR related to that
7 found in local humans and livestock, AMR is still common in remote wildlife
8 populations with little direct human influence. Most reports of AMR in wildlife are
9 survey based and/or small scale, so researchers can only speculate on possible
10 sources and sinks of AMR or the impact of wildlife AMR on clinical resistance. This
11 lack of quantitative data on the flow of antimicrobial resistance genes and AMR
12 bacteria across the natural environment could reflect the numerous AMR sources
13 and amplifiers in the populated world. Ecosystems with relatively simple and well
14 characterised potential inputs of AMR can provide tractable, but realistic, systems
15 for studying AMR in the natural environment. New tools, such as animal tracking
16 technologies and high-throughput sequencing of resistance genes and mobilomes,
17 should be integrated with existing methodologies to understand how wildlife
18 maintains and disperses AMR.

1 ***Introduction***

2 A growing human population and increasing fragmentation of natural habitats
3 inevitably forces wildlife into greater contact, both direct and indirect, with humans
4 and their livestock, thereby increasing the opportunities for transmission of infection
5 between and within populations [1]. While some progress has been made in
6 understanding the epidemiology of multi-host infections involving wildlife [2], less
7 attention has been paid to the role of wild animals in the ecology and evolution of
8 antimicrobial resistance (AMR) [3, 4]. Although AMR is considered one of the
9 greatest challenges to global health security [5], to date, most AMR research has
10 been based in clinical settings [6]. Relatively little is known about the flow and fate of
11 AMR in the natural environment [7], particularly in highly mobile species that could
12 act as efficient AMR dispersers [3, 4] (Figure 1). In this review, we discuss the
13 possible role of wildlife in the dissemination of AMR, specifically how wildlife might
14 acquire and transport AMR and the potential for them to transmit AMR to humans
15 and livestock.

16

17 ***Antimicrobial Resistance***

18 Antimicrobial drugs have saved millions of human lives and improved animal health
19 and welfare globally [6]. Consequently, the evolution and dispersal of AMR is
20 considered to be a major problem facing medical science and food security [5]. AMR
21 is an ancient phenomenon, having evolved in dynamic microbial communities within
22 which antimicrobials are produced by environmental bacteria and fungi naturally
23 living in soil, water etc [6]. Such AMR, plus AMR as a side effect of selection of other
24 properties, including efflux pumps for removing environmental stressors such as

1 heavy metals, is often referred to as 'intrinsic' AMR. In contrast, 'acquired' AMR is
2 the result of exposure to antimicrobial drugs which promotes resistance by selecting
3 bacteria within a population with genetic traits conferring resistance. Thus, the
4 selection of AMR in both pathogens and the normal gut microbiota of livestock and
5 humans is believed to be largely a consequence of increased selective pressure
6 provided by clinical antimicrobial use: recent hospitalisation, for example, is a risk
7 factor for shedding antibiotic resistant *E. coli* in both horses [8] and humans [9]. In
8 many parts of the world, antimicrobials are still used, not just in clinical settings, but
9 as 'growth promoters' in food-producing animals, an activity banned in the EU owing
10 to concerns about the selection of AMR [10]. So, while wildlife could provide a
11 reservoir of intrinsic genetic determinants for resistance, it has usually been
12 assumed that AMR detected in wildlife samples is acquired AMR resulting directly or
13 indirectly from antibiotic-treated humans or livestock [11].

14 The ecology of AMR is complicated by the horizontal spread of the genes
15 encoding AMR through communities of different species and even genera of
16 bacteria, via mobile genetic elements such as plasmids (extra-chromosomal DNA
17 molecules). These mobile genetic elements often encode multiple genes, providing
18 resistance to antimicrobials and, indeed, other environmental chemical stressors
19 including metals and disinfectants. Consequently exposure to one antimicrobial (or
20 other stressor) can select for all co-encoded genes and thus the rapid emergence of
21 multi-drug resistance [6]. Thus, wildlife and other environmental bacteria that have
22 never been found to infect humans can, through horizontal gene transfer, exchange
23 resistance mechanisms with human pathogens [11, 12] (but see [13]).

24

1

2 *Potential sources of AMR in the environment to wildlife*

3 Following selection of resistance within individuals (human or domesticated animals)
4 treated with antimicrobials [10], both resistant bacteria and antimicrobials are
5 subsequently excreted by the patient (Figure 1). These can be dispersed in the
6 environment, for example in sewage effluent pumped into rivers [14] and spreading
7 sewage sludge as a fertiliser, or in the faeces of treated livestock and pets [15,
8 16](Figure 2). Effluent and run-off from fields will often end up flowing into the sea,
9 resulting in estuaries, coastal waters and beaches polluted by faecal matter
10 [14](Figure 1). This could be a critical point of contact where humans and marine
11 animals, as well as waders and seabirds, are exposed to AMR [17]. The rapidly
12 expanding aquaculture industry is another source of AMR and antimicrobials to the
13 environment: fish and seafood farmed in some countries where antimicrobial usage
14 is high and poorly regulated are particularly likely to carry medically significant
15 resistant pathogens [4, 18].

16 Evolution of AMR does not necessarily stop in the gastro-intestinal tract of
17 animals (including humans) undergoing treatment; many antimicrobials can be
18 excreted in an active form and persist in the environment [19]. Thus ongoing
19 exposure to antimicrobial drugs, for example in sewage, might maintain the selective
20 advantage of AMR and promote the proliferation of resistance determinants and
21 resistant bacteria in the environment. There is an added risk from sites highly
22 contaminated with excreta, such as intensive farms and sewage treatment plants.
23 Places with a high abundance and diversity of bacteria provide a high density of
24 bacterial hosts and excellent conditions for the horizontal transmission of

1 antimicrobial resistance genes from commensal or environmental to pathogenic
2 bacteria [20]. It is clear that, particularly in areas with dense human or livestock
3 populations, there is a myriad of AMR sources and amplifiers. If AMR genes and
4 bacteria are carried in the gut of wildlife, then coupled with inadequate waste
5 management and long-range animal movements, there is potential for wildlife to
6 transport new and emerging antimicrobial resistance genes around the world [14]
7 (Figure 1).

8

9 *Patterns of AMR infection in wildlife*

10 With increasing pressure from expanding human populations, wild animals are
11 increasingly forced to forage on resources contaminated by human 'pathogen
12 pollution' [2, 14]. So it is not surprising that AMR has often been described in peri-
13 domestic wildlife [11]. AMR has been detected, particularly among commensal gut
14 bacteria, in wild mammals, birds, reptiles and fish, with the prevalence and
15 resistance patterns varying across species, locations and possibly time (e.g. [3, 18,
16 21-25]). Current data on AMR in wildlife largely consist of series of 'snap shots'
17 proving the presence of resistomes (all of the antibiotic resistance genes found in
18 microbes [13]) in those animals, but little else. However, the few studies that identify
19 potential sources of AMR and can make comparisons across sites differing in
20 contamination provide insights into the potential for wildlife to disseminate clinically
21 relevant AMR.

22 Studies in South America and Africa, found AMR to be more common in gut
23 bacteria from non-human primates living close to humans than in those from more
24 isolated populations [26, 27]. Ugandan gorilla populations, for example, with home

1 ranges that overlapped human settlements harboured resistant bacteria that were
2 genetically similar to *E. coli* from those people and livestock, compared with apes
3 more remotely located [26]. In Northern elephant seals, *Mirounga angustirostris*, the
4 probability of shedding antimicrobial resistant *E. coli* was found to be directly
5 correlated with the size of local human populations [28]. Similarly, in the Galapagos,
6 molecular markers of AMR were more common in both seawater samples and
7 marine iguanas close to tourist sites compared with those from more pristine
8 conservation areas [23]. There are, however, exceptions to the generally positive
9 relationship between spatial distance to anthropogenic wastes and the detection of
10 clinically important resistance genes. For example, resistance to ciprofloxacin, a
11 relatively recently developed and completely synthetic antimicrobial, was detected
12 even in the most remote groups of monkeys in Mexico [27]. This is suggestive of *de*
13 *novo* evolution of resistance, horizontal gene transfer from environmental microbes
14 and/or greater contact with humans than previously thought. Further molecular and
15 ecological investigations are clearly required. In general, however, study sites with
16 relatively low or well defined AMR inputs enable us to quantify spatial patterns,
17 pathways and processes that drive AMR dissemination at different scales.

18 In heavily populated areas, high background AMR levels often cloud
19 observations. In the UK, for example, we and others have found that AMR is
20 frequently found in both wild mammals and birds, although the sources and drivers
21 of AMR are often unclear [22, 29-31]; see Fig. 2 and Table 1]. We found that the
22 patterns of AMR in *E. coli* from the livestock and rodents resident on intensive
23 livestock farms (Table 1), and the genes encoding that resistance, were often similar.
24 The *E. coli* only rarely identified shared genotypes, however, suggesting an important

1 role for the mobilome (all mobile genetic elements in a genome, e.g. plasmids) and
2 horizontal transmission of AMR rather than simple cross-species transmission of
3 resistant bacteria. In contrast, at less intensively farmed sites, such as dairy farms
4 with cattle kept outside, no clear relationships between either patterns or the
5 genetics of AMR were found in livestock and wildlife [31]. As in the African and South
6 American studies, we also found AMR in wildlife in relatively remote and
7 uninhabited (by humans) areas (Table 1). Furthermore, sympatric populations of wild
8 mammals, including different species of rodents sharing the same woodland habitat,
9 had different patterns of AMR and/or different temporal dynamics [29]. This strongly
10 suggests that AMR in the bacterial microbiota of wildlife is not simply a matter of
11 recent anthropogenic contamination or selection.

12 So while most studies in wildlife have assumed that AMR in wildlife is the
13 consequence of spill-over of resistant bacteria from domestic animals or people [32,
14 33], there are several non-exclusive alternative hypotheses that challenge this notion
15 of recent transmission. For example, following exposure to wastes containing
16 pharmaceuticals, enteric bacteria present in wildlife evolve resistance through
17 selection of pre-existing environmental antimicrobial resistance genes. These might
18 become 'naturalised' in the gut microbiota, but also AMR genes (which have been
19 found in ancient environmental samples [13]) are, and have always been, a normal
20 finding in commensal gut microbiota. Moreover, distinguishing between AMR
21 recently acquired from anthropogenic sources, such as a farm or sewage treatment
22 plants, and 'intrinsic' (or at least 'naturalised') background AMR will be challenging.
23 Comparing the similarity of sequences of resistance genes collected from sites

1 differing in their connectedness to sources of acquired AMR (e.g. using sequence
2 similarity network approaches [12]) could provide the evidence required.

3 A particular concern about AMR dispersal is wildlife species that have the
4 capacity for long range movements. Migratory birds arriving from beyond national
5 boundaries could transfer new or emerging patterns of AMR, but even resident
6 species have the potential to move AMR from hotspots to vulnerable populations.
7 The potential of wild animals to disseminate AMR depends on their AMR 'infection'
8 status, their direct and indirect contact with other populations and their movements
9 within the landscape. In communal corvid roosts in Europe and the USA, 2.5 – 6.0%
10 of faecal samples contained resistance genes for vancomycin, a antimicrobial 'of last
11 resort' in human medicine [21, 34]. Gulls carrying medically significant AMR, are
12 capable of long range movements and are increasingly found feeding on
13 anthropogenic waste and nesting in urban areas [22], [25, 35]. Similarly, in aquatic
14 ecosystems uneaten food and faeces from human sewage, agriculture and
15 aquaculture containing antimicrobials and AMR bacteria can be ingested by wild fish
16 and other organisms, which can travel enormous distances and in some cases enter
17 the human food chain [18]. However, most of these studies on globally moving
18 species are one-off surveys of AMR prevalence with no attempt to identify infection
19 sources (or sinks) [3], which limits our ability to estimate the risk posed by migratory
20 species in disseminating AMR. Finally, it remains unknown whether AMR can be or,
21 more importantly, is transmitted from wildlife to humans or domestic animals, which
22 is the main concern of clinicians and policy makers.

23

24 *Studying AMR dispersal by wildlife*

1 Given the many knowledge gaps, a range of tools and approaches will be
2 needed to identify and characterise transmission routes of AMR in wildlife. At a
3 broad scale, identifying traits that predispose wildlife species or functional groups to
4 transmit AMR could be determined by integrating ecological, biological and life
5 history datasets for vertebrate hosts with metagenome sequences embedding
6 resistance determinants [12]. While this is an efficient and informative approach,
7 one caveat is that by mining such data, we can only find known resistance
8 determinants. Some evidence from wildlife studies shows that the genes
9 responsible for phenotypic resistance are often not detectable using PCRs targeted
10 at common clinical AMR genes. This suggests a greater diversity of resistance genes
11 (many of which will already have been associated with other, non-AMR, functions) in
12 the environment than found in clinical isolates (Authors' Unpub. Data).

13 At a finer scale, study systems are needed in which clear and measurable
14 transmission routes for AMR exist and the movement of wildlife can be tracked. The
15 discovery of multidrug resistance in species of high conservation value on oceanic
16 islands [23] and in samples from isolated, relatively untouched points on continents
17 [25] provide 'natural experiments' that are ideal for studying patterns and processes
18 in the ecology and evolution of AMR. Monitoring AMR genes within such pristine
19 ecosystems (e.g. Arctic or nature reserves with tight biosecurity), or at their interface
20 with human-influenced areas enables us to estimate the frequency with which genes
21 encoding resistance are exchanged in microbial communities. Such microbial
22 communities can exist within human, domestic animal and wildlife populations, as
23 well as the wider environment [25, 36].

1 When working in the more contaminated ‘natural’ environments common to
2 densely populated areas, distinguishing between AMR acquired from anthropogenic
3 sources, such as a farm or sewage treatment plant, versus naturally occurring or
4 naturalised ‘background’ AMR will be more challenging. One approach is to study the
5 dispersal of relatively rare AMR determinants, currently associated only with human
6 (or particular livestock) populations, through food chains. For example,
7 fluoroquinolone resistance and extended-spectrum beta-lactamases (ESBL)
8 (conferring resistance to newer antibiotics used in human medicine), are relatively
9 unusual in livestock and, in our experience, incredibly rare in wildlife. Such resistance
10 might be tracked through high risk ecosystems, for example from sewage treatment
11 plants or livestock slurry pits into the surrounding environment, at multiple levels:
12 phenotypic resistance, bacterial genotype, mobile elements and individual resistance
13 genes. High-throughput next-generation sequencing, can rapidly provide such
14 detailed forensic trails [13]. Although targeted at a limited range of AMR, this
15 approach would provide a good understanding of the ecology of AMR genes and
16 their ‘resistome’ context. Deeper, meta-genomic sequencing studies through these
17 and/or less high risk ecosystems will be needed to place such targeted AMR studies
18 in a broader perspective, through examining a range of AMR genes across taxa of
19 host bacteria within the same samples. However, metagenomic studies have their
20 own challenges, not least the volume and complexity of bioinformatic data analysis
21 and the cost, which currently limits sample number and interpretation.

22 Ecological models of AMR transmission involving wildlife need to incorporate
23 indirect rather than just direct host-to-host transmission. Although AMR can be
24 transmitted directly between hosts, for example through predation (food-borne in a

1 clinical context) or grooming and faeco-oral transmission, there is a huge overlap
2 between the microbiota of the normal gut and that of the external environment
3 (e.g. in soil and water) with horizontal transmission of AMR possible in both. Such
4 models could be based on spatial movements in relation to a common
5 environmental source of AMR contamination such as a refuse dump [26]. Sewage
6 treatment plants, for example, are hotspots of AMR, which can provide valuable
7 pockets of semi-natural habitat for birds and bats, attracted by the invertebrates
8 that themselves feed in the sewage [37]. In fragmented landscapes, birds and bats
9 often then move between isolated discrete patches of suitable habitat or food
10 sources [38], such as gardens and farms, enabling the further dispersal of AMR. Ever
11 more powerful and accurate electronic tracking devices and spatial modelling
12 approaches provide the potential to map the movements of animals in both space
13 and time relative to potential sources of AMR pollution and points of contact with
14 humans and livestock [39]. By combining a range of tools including mark-recapture
15 methods, epidemiological modelling, molecular sequencing, behavioural
16 observations and high tech devices such as GPS trackers, we can start to test
17 empirically hypotheses concerning the dissemination of AMR by wildlife.

18

19 *Consequences of AMR for wildlife*

20 The consequences for wildlife of the evolution of AMR in commensal, or even
21 pathogenic, bacteria are untested [36], but probably small. Unlike avian influenza
22 [40], for example, AMR is not a disease and does not appear to reduce the survival or
23 dispersal capacity of 'infected' animals, although this has not been explicitly tested.

24 The clinical issue with AMR in both human and livestock populations is not that it

1 causes disease, but that it threatens the ability to treat infections with
2 antimicrobials, a practice rare in wild-living populations. AMR could compromise the
3 treatment of individual wild animals in captivity, e.g. in wildlife hospitals, or of highly
4 managed populations , especially those immuno-compromised due to low genetic
5 diversity (e.g. [41]). This might be exacerbated by conservation management
6 measures such as translocation of rare species that could expedite the spread of
7 novel microbes or antimicrobial genes between isolated populations [42].

8 The biggest issue for wildlife populations is the management response should
9 they be thought to be significant sources of AMR for humans or livestock (see also
10 Table 2). The control of wildlife infections transmissible to humans and livestock
11 relies on three main approaches – separation of, or at least reducing contact with,
12 the wildlife source, vaccination, and wildlife population control, often by culling.
13 Vaccination is not possible for AMR control, and the physical separation of wildlife
14 from livestock is difficult, expensive and, except very locally (e.g. keeping rodents or
15 birds out of feed stores), impracticable. Protecting the human food chain from AMR
16 is important but challenging given that wild game, seafood and bushmeat are
17 important both nutritionally and culturally in many human societies [4].
18 Furthermore, control and mitigation measures such as improved hygiene and
19 restriction on movements cannot be easily implemented, if at all, for free living
20 animals. For logistical, economic, historical and cultural reasons, culling is often the
21 approach taken: however, the efficacy and efficiency of culling wildlife in controlling
22 disease are at best controversial.

23

24 *Research and policy priorities*

1 Wildlife clearly is shedding and therefore able to disseminate AMR [2, 4]. However,
2 few studies have identified the likely selection factors (including, but not necessarily
3 limited to, sources of antimicrobial exposure), origins of the resistance genes, or,
4 importantly, the direction of transmission. Studying infection transmission in wildlife
5 poses a number of challenges, particularly for a complex issue such as AMR that is
6 present in, and can move between, multiple bacterial taxa in multiple hosts and the
7 environment. Approaches used to study and control AMR transmission in the clinical
8 setting are challenging to apply to wildlife systems. Contact between wildlife and
9 sources of AMR and/or antimicrobials often cannot be measured directly but need to
10 be inferred, for example from molecular ‘fingerprints’ of specific contamination. This
11 can be supplemented with behavioural observations and electronic tracking devices
12 fitted to wild animals.

13 Interventions that minimise and mitigate the transmission of AMR from
14 livestock or human populations to wildlife need researching alongside investigation
15 of the risk itself, in order to develop both evidence-based and proportionate
16 protocols and policies (Table 2). Pollution control and sewage treatment are likely
17 priority areas for such research, particular in countries with few controls on either
18 antibiotic usage or release of untreated wastes (which includes both developed and
19 developing countries). Meanwhile ecologists studying wild populations, along with
20 wildlife hospitals and existing programmes designed to monitor pollution, poisoning
21 and diseases in wildlife (e.g. WILDCOMS [43]), might be recruited to collect samples
22 for surveillance. This last approach might be particularly useful in identifying species,
23 key individuals within populations or spatial locations that are ‘super spreaders’ of

1 AMR transmission and could be targeted for focused surveillance, control or
2 mitigation measures [39].

3 It is important to study AMR in wildlife as a potential hazard to human health
4 and food security, especially given that about 40% of emerging human diseases are
5 thought to have originated in wildlife [1]. Tropical ecosystems and areas in which
6 humans live close to both livestock and wildlife are likely to present heightened, but
7 to date poorly studied, risks for the evolution and transmission of AMR by wildlife
8 (Table 2). Furthermore, studies of AMR in wildlife can have wider impact than simply
9 public health risk. First, by stepping outside of the 'blame game' of livestock,
10 veterinary and medical systems they can elucidate fundamental issues in the
11 evolution and transmission ecology of antimicrobial resistant bacteria and resistance
12 determinants that can be applied back into more clinical settings. Second, a better
13 understanding of the role of wildlife in AMR dissemination should help us decide if
14 control and mitigation strategies are required and where best to apply them. Finally,
15 while wildlife might be long distance dispersers of AMR, they can also be sentinels
16 for the abundance and distribution of pathogen pollution in our environment.

17

18 **Ethics**

19 All work carried out conformed to the legal requirements of the country in which the
20 work was carried out and to all institutional guidelines.

21

22 **Competing Interests**

23 We have no competing interests.

24

1 **Authors' contributions**

2 All authors contributed to the writing of this review and gave final approval for
3 publication.

4

5 **Acknowledgements**

6 Thank you to D. Graham for discussions about AMR and to L. Al Meslati for
7 permission to reproduce a figure and part of a table from his PhD thesis. Some of the
8 data shown in figure 2 and table 1 were collected through Defra-funded research
9 projects.

10

11 **Funding**

12 No specific funding was provided for the preparation of this paper.

13

1 References

- 2 [1] Jones, K.E., Patel, N.G., Levy, M.A., Storeygard, A., Balk, D., Gittleman, J.L. Daszak,
3 P. 2008 Global trends in emerging infectious diseases. *Nature* **451**, 990-993.
4 (doi:10.1038/nature06536).
- 5 [2] Wiethoelter, A.K., Beltrán-Alcrudo, D., Kock, R. & Mor, S.M. 2015 Global trends in
6 infectious diseases at the wildlife–livestock interface. *Proceedings of the National*
7 *Academy of Sciences* **112**, 9662-9667. (doi:10.1073/pnas.1422741112).
- 8 [3] Huijbers, P.M.C., Blaak, H., de Jong, M.C.M., Graat, E.A.M., Vandenbroucke-
9 Grauls, C.M.J.E. & Husman, A.M.d.R. 2015 Role of the Environment in the
10 Transmission of Antimicrobial Resistance to Humans: A Review. *Environmental*
11 *Science & Technology* **49**, 11993-12004. (doi:10.1021/acs.est.5b02566).
- 12 [4] Greig, J., Rajic, A., Young, I., Mascarenhas, M., Waddell, L. & LeJeune, J. 2015 A
13 Scoping Review of the Role of Wildlife in the Transmission of Bacterial Pathogens and
14 Antimicrobial Resistance to the Food Chain. *Zoonoses and Public Health* **62**, 269-284.
15 (doi:10.1111/zph.12147).
- 16 [5] WHO. 2014 Antimicrobial resistance: global report on surveillance 2014. (World
17 Health Organisation).
- 18 [6] Davies, J. & Davies, D. 2010 Origins and Evolution of Antibiotic Resistance.
19 *Microbiology and Molecular Biology Reviews* **74**, 417-+. (doi:10.1128/mubr.00016-
20 10).
- 21 [7] Wellington, E.M.H., Boxall, A.B.A., Cross, P., Feil, E.J., Gaze, W.H., Hawkey, P.M.,
22 Johnson-Rollings, A.S., Jones, D.L., Lee, N.M., Otten, W., et al. 2013 The role of the
23 natural environment in the emergence of antibiotic resistance in Gram-negative
24 bacteria. *Lancet Infectious Diseases* **13**, 155-165.
- 25 [8] Maddox, T.W., Pinchbeck, G.L., Clegg, P.D., Wedley, A.L., Dawson, S. & Williams,
26 N.J. 2012 Cross-sectional study of antimicrobial-resistant bacteria in horses. Part 2:
27 Risk factors for faecal carriage of antimicrobial-resistant *Escherichia coli* in horses.
28 *Equine veterinary journal* **44**, 297-303. (doi:10.1111/j.2042-3306.2011.00440.x).
- 29 [9] Cardoso, T., Ribeiro, O., Aragao, I.C., Costa-Pereira, A. & Sarmiento, A.E. 2012
30 Additional risk factors for infection by multidrug-resistant pathogens in healthcare-
31 associated infection: a large cohort study. *Bmc Infectious Diseases* **12**.
32 (doi:10.1186/1471-2334-12-375).
- 33 [10] Marshall, B.M. & Levy, S.B. 2011 Food Animals and Antimicrobials: Impacts on
34 Human Health. *Clinical Microbiology Reviews* **24**, 718-733. (doi:10.1128/cmr.00002-
35 11).
- 36 [11] Vittecoq, M., Godreuil, S., Prugnotte, F., Durand, P., Brazier, L., Renaud, N., Arnal,
37 A., Aberkane, S., Jean-Pierre, H., Gauthier-Clerc, M., et al. 2016 REVIEW:
38 Antimicrobial resistance in wildlife. *Journal of Applied Ecology* **53**, 519-529.
39 (doi:10.1111/1365-2664.12596).
- 40 [12] Fondi, M., Karkman, A., Tamminen, M., Bosi, E., Virta, M., Fani, R., Alm, E. &
41 McInerney, J. 2016 Every gene is everywhere but the environment selects: Global
42 geo-localization of gene sharing in environmental samples through network analysis.
43 *Genome Biology and Evolution*. (doi:10.1093/gbe/evw077).
- 44 [13] Forsberg, K.J., Patel, S., Gibson, M.K., Lauber, C.L., Knight, R., Fierer, N. & Dantas,
45 G. 2014 Bacterial phylogeny structures soil resistomes across habitats. *Nature* **509**,
46 612-616. (doi:10.1038/nature13377).

1 [14] Graham, D.W., Collignon, P., Davies, J., Larsson, D.G.J. & Snape, J. 2014
2 Underappreciated Role of Regionally Poor Water Quality on Globally Increasing
3 Antibiotic Resistance. *Environmental Science & Technology* **48**, 11746-11747.
4 (doi:10.1021/es504206x).

5 [15] Leatherbarrow, A.J.H., Griffiths, R., Hart, C.A., Kemp, R., Williams, N.J., Diggle,
6 P.J., Wright, E.J., Sutherst, J., Houghton, P. & French, N.P. 2007 *Campylobacter lari*:
7 genotype and antibiotic resistance of isolates from cattle, wildlife and water in an
8 area of mixed dairy farmland in the United Kingdom. *Environmental Microbiology* **9**,
9 1772-1779. (doi:10.1111/j.1462-2920.2007.01295.x).

10 [16] Maddox, T.W., Williams, N.J., Clegg, P.D., O'Donnell, A.J., Dawson, S. &
11 Pinchbeck, G.L. 2011 Longitudinal study of antimicrobial-resistant commensal
12 *Escherichia coli* in the faeces of horses in an equine hospital. *Preventive Veterinary*
13 *Medicine* **100**, 134-145. (doi:10.1016/j.prevetmed.2011.02.006).

14 [17] Leonard, A.F.C., Zhang, L., Balfour, A.J., Garside, R. & Gaze, W.H. 2015 Human
15 recreational exposure to antibiotic resistant bacteria in coastal bathing waters.
16 *Environment International* **82**, 92-100.
17 (doi:<http://dx.doi.org/10.1016/j.envint.2015.02.013>).

18 [18] Cabello, F.C., Godfrey, H.P., Tomova, A., Ivanova, L., Dölz, H., Millanao, A. &
19 Buschmann, A.H. 2013 Antimicrobial use in aquaculture re-examined: its relevance
20 to antimicrobial resistance and to animal and human health. *Environmental*
21 *Microbiology* **15**, 1917-1942. (doi:10.1111/1462-2920.12134).

22 [19] Kasprzyk-Hordern, B., Dinsdale, R.M. & Guwy, A.J. 2009 The removal of
23 pharmaceuticals, personal care products, endocrine disruptors and illicit drugs
24 during wastewater treatment and its impact on the quality of receiving waters.
25 *Water Research* **43**, 363-380. (doi:<http://dx.doi.org/10.1016/j.watres.2008.10.047>).

26 [20] Heuer, H., Schmitt, H. & Smalla, K. 2011 Antibiotic resistance gene spread due to
27 manure application on agricultural fields. *Current Opinion in Microbiology* **14**, 236-
28 243. (doi:10.1016/j.mib.2011.04.009).

29 [21] Oravcova, V., Ghosh, A., Zurek, L., Bardon, J., Guenther, S., Cizek, A. & Literak, I.
30 2013 Vancomycin-resistant enterococci in rooks (*Corvus frugilegus*) wintering
31 throughout Europe. *Environmental Microbiology* **15**, 548-556. (doi:10.1111/1462-
32 2920.12002).

33 [22] Bonnedahl, J. & Järhult, J.D. 2014 Antibiotic resistance in wild birds. *Upsala*
34 *Journal of Medical Sciences* **119**, 113-116. (doi:doi:10.3109/03009734.2014.905663).

35 [23] Wheeler, E., Hong, P.-Y., Bedon, L.C. & Mackie, R.I. 2012 Carriage of antibiotic-
36 resistant enteric bacteria varies among sites in Galapagos reptiles. *Journal of Wildlife*
37 *Diseases* **48**, 56-67.

38 [24] Gilliver, M.A., Bennett, M., Begon, M., Hazel, S.M. & Hart, C.A. 1999
39 Enterobacteria - Antibiotic resistance found in wild rodents. *Nature* **401**, 233-234.

40 [25] Sjölund, M., Bonnedahl, J., Hernandez, J., Bengtsson, S., Cederbrant, G.,
41 Pinhassi, J., Kahlmeter, G. & Olsen, B. 2008 Dissemination of Multidrug-Resistant
42 Bacteria into the Arctic. *Emerging Infectious Diseases* **14**, 70-72.
43 (doi:10.3201/eid1401.070704).

44 [26] Rwego, I.B., Isabirye-Basuta, G., Gillespie, T.R. & Goldberg, T.L. 2008
45 Gastrointestinal Bacterial Transmission among Humans, Mountain Gorillas, and
46 Livestock in Bwindi Impenetrable National Park, Uganda. *Conservation Biology* **22**,
47 1600-1607. (doi:10.1111/j.1523-1739.2008.01018.x).

- 1 [27] Cristóbal-Azkarate, J., Dunn, J.C., Day, J.M.W. & Amábile-Cuevas, C.F. 2014
2 Resistance to Antibiotics of Clinical Relevance in the Fecal Microbiota of Mexican
3 Wildlife. *PLoS ONE* **9**, e107719. (doi:10.1371/journal.pone.0107719).
- 4 [28] Stoddard, R.A., Atwill, E.R., Gulland, F.M.D., Miller, M.A., Dabritz, H.A., Paradies,
5 D.M., Worcester, K.R., Jang, S., Lawrence, J., Byrne, B.A., et al. 2008 Risk factors for
6 infection with pathogenic and antimicrobial-resistant fecal bacteria in northern
7 elephant seals in California. *Public Health Rep.* **123**, 360-370.
- 8 [29] Williams, N.J., Sherlock, C., Jones, T.R., Clough, H.E., Telfer, S.E., Begon, M.,
9 French, N., Hart, C.A. & Bennett, M. 2011 The prevalence of antimicrobial-resistant
10 *Escherichia coli* in sympatric wild rodents varies by season and host. *Journal of*
11 *Applied Microbiology* **110**, 962-970. (doi:10.1111/j.1365-2672.2011.04952.x).
- 12 [30] Williams, N.J., Hunt, D., Jones, T.R., French, N.P., Begon, M., Bennett, M. & Hart,
13 C.A. 2002 Antibiotic resistance in bank voles (*Clethrionomys glareolus*) and wood
14 mice (*Apodemus sylvaticus*). *Research in Veterinary Science* **72**, 40-40.
15 (doi:10.1016/s0034-5288(02)90113-x).
- 16 [31] Al-Tunesi, L.A. 2009 Prevalence of antibiotic-resistant *Escherichia coli* in faecal
17 samples from domestic animals and wildlife : a cross-sectional study, University of
18 Liverpool.
- 19 [32] Wardyn, S.E., Kauffman, L.K. & Smith, T.C. 2012 Methicillin-resistant
20 *Staphylococcus aureus* in Central Iowa Wildlife. *Journal of Wildlife Diseases* **48**, 1069-
21 1073. (doi:10.7589/2011-10-295).
- 22 [33] Porrero, M.C., Mentaberre, G., Sánchez, S., Fernández-Llario, P., Gómez-Barrero,
23 S., Navarro-Gonzalez, N., Serrano, E., Casas-Díaz, E., Marco, I., Fernández-Garayzabal,
24 J.-F., et al. 2013 Methicillin resistant *Staphylococcus aureus* (MRSA) carriage in
25 different free-living wild animal species in Spain. *The Veterinary Journal* **198**, 127-
26 130. (doi:<http://dx.doi.org/10.1016/j.tvjl.2013.06.004>).
- 27 [34] Oravcova, V., Zurek, L., Townsend, A., Clark, A.B., Ellis, J.C., Cizek, A. & Literak, I.
28 2013 American crows as carriers of vancomycin-resistant enterococci with vanA
29 gene. *Environmental Microbiology* **16**, 939-49 . (doi:10.1111/1462-2920.12213).
- 30 [35] Stedt, J., Bonnedahl, J., Hernandez, J., McMahon, B.J., Hasan, B., Olsen, B.,
31 Drobni, M. & Waldenström, J. 2014 Antibiotic resistance patterns in *Escherichia coli*
32 from gulls in nine European countries. *Infection Ecology & Epidemiology* **4**,
33 10.3402/iee.v3404.21565. (doi:10.3402/iee.v4.21565).
- 34 [36] Albrechtova, K., Papousek, I., De Nys, H., Pauly, M., Anoh, E., Mossoun, A.,
35 Dolejska, M., Masarikova, M., Metzger, S., Couacy-Hymann, E., et al. 2014 Low Rates
36 of Antimicrobial-Resistant Enterobacteriaceae in Wildlife in Tai National Park, Cote
37 d'Ivoire, Surrounded by Villages with High Prevalence of Multiresistant ESBL-
38 Producing *Escherichia coli* in People and Domestic Animals. *Plos One* **9**.
39 (doi:10.1371/journal.pone.0113548).
- 40 [37] Park, K.J. & Cristinacce, A. 2006 Use of sewage treatment works as foraging sites
41 by insectivorous bats. *Animal Conservation* **9**, 259-268.
- 42 [38] Lawton, J.H., Brotherton, P.N.M., Brown, V.K., Elphick, C., Fitter, A.H., Forshaw,
43 J., Haddow, R.W., Hilborne, S., Leafe, R.N., Mace, G.M., Southgate, M.P., Sutherland,
44 W.A., & Tew, T.E., Varley, J., & Wynne, G.R. . 2010 Making Space for Nature: a
45 review of England's wildlife sites and ecological network. (UK, Defra).

1 [39] Craft, M.E. & Caillaud, D. 2011 Network Models: An Underutilized Tool in
2 Wildlife Epidemiology? *Interdisciplinary Perspectives on Infectious Diseases* **2011**, 12.
3 (doi:10.1155/2011/676949).

4 [40] Feare, C.J. 2010 Role of Wild Birds in the Spread of Highly Pathogenic Avian
5 Influenza Virus H5N1 and Implications for Global Surveillance. *Avian Diseases* **54**,
6 201-212. (doi:10.1637/8766-033109-ResNote.1).

7 [41] Morris, K., Austin, J.J. & Belov, K. 2013 Low major histocompatibility complex
8 diversity in the Tasmanian devil predates European settlement and may explain
9 susceptibility to disease epidemics. *Biology Letters* **9**. (doi:10.1098/rsbl.2012.0900).

10 [42] Grange, Z.L., Gartrell, B.D., Biggs, P.J., Nelson, N.J., Marshall, J.C., Howe, L., Balm,
11 M.G.M. & French, N.P. 2015 Using a common commensal bacterium in endangered
12 Takahe as a model to explore pathogen dynamics in isolated wildlife populations.
13 *Conservation Biology* **29**, 1327-1336. (doi:10.1111/cobi.12521).

14 [43] Pereira, M.G., Chaplow, J.S. & F., S.R. 2015 WILDCOMS (Wildlife Disease &
15 Contaminant Monitoring and Surveillance network) annual report 2013-2014. (p. 19.
16 Lancaster, UK, NERC/Centre for Ecology & Hydrology.

17 [44] Anon. 2007 BSAC Methods for Antimicrobial Susceptibility Testing (Version 6.1).
18 Birmingham, BRITISH SOCIETY OF ANTIMICROBIAL CHEMOTHERAPY
19
20
21

1 **Figure and Table Captions**

2 Figure 1: Dispersal of AMR across the landscape: between human communities,
3 hospitals, sewage treatment plants (STPs), farms and the wider environment
4 including via wildlife (Modified from [6]).

5

6 Figure 2: Antimicrobial resistance in wildlife on dairy farms in Cheshire, UK. The
7 resistance patterns of *E.coli* from the faeces of cattle, rodents (mainly *Myodes*
8 *glaruelus* and *Apodemus sylvaticus*), wild birds (mainly passerines) and other wild
9 mammals (mainly badgers and foxes) were compared: 1A) Percentage of faecal
10 samples containing *E. coli* resistant to at least one antibiotic on five different farms
11 (A-F). 1B) Percentage of *E. coli* isolated from each group of animals resistant to
12 various antibiotics or multidrug resistant. Resistance to the following antibiotics was
13 tested: ampicillin (amp), chloramphenicol (chl), tetracycline (tet), trimethoprim (trm)
14 and nalidixic acid (nal) and also *MDR (multi-drug resistance defined as resistance to
15 three or more of the antibiotics tested). All susceptibility testing was performed
16 according to the British Society of Antimicrobial Chemotherapy guidelines [44].
17 Figure modified from [31].

18

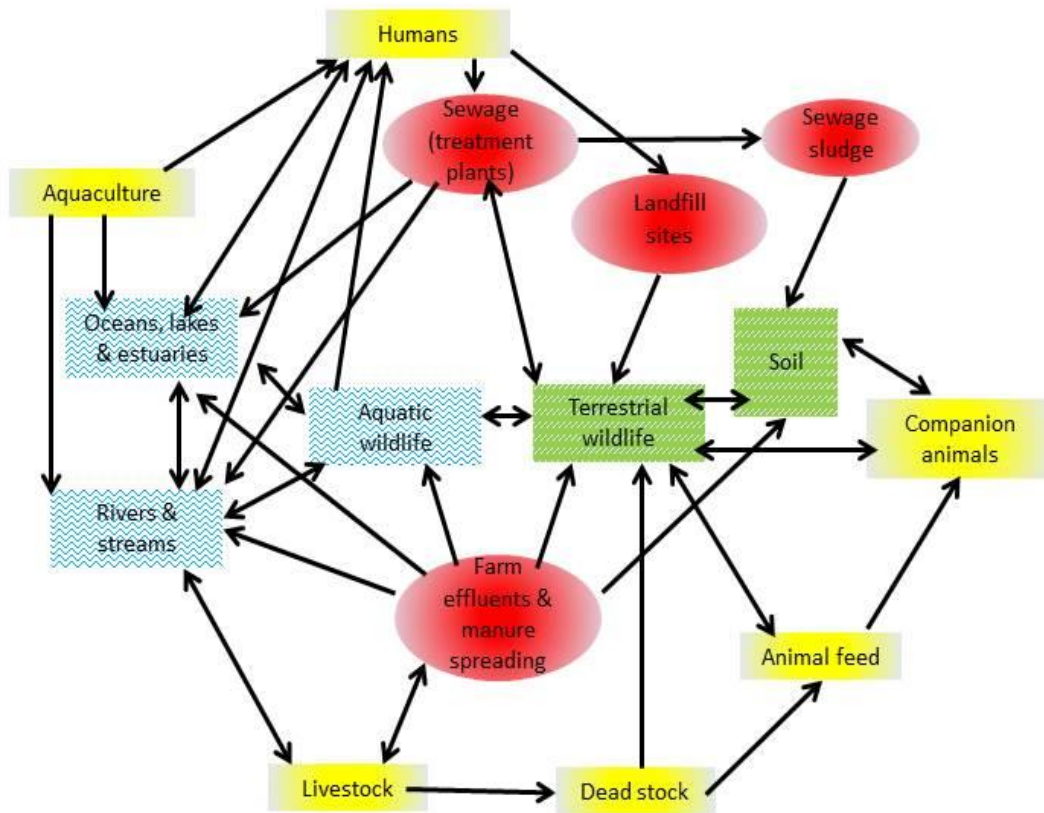
19 Table 1: Antimicrobial resistant *E. coli* in the faeces of wild rodents collected at sites
20 in the UK varying in predicted exposure to livestock treated with antimicrobial drugs.
21 Resistance to six antibiotics (ampicillin (amp), apramycin (apr), chloramphenicol
22 (chl), tetracycline (tet), trimethoprim (trm) and nalidixic acid (nal)) was investigated
23 [44]. Modified from [31]

24

1 Table 2: Summary of some of the key outstanding questions, mitigation measures
2 and research approaches regarding the role of wildlife in the transmission of AMR
3 based on the literature reviewed. Suggested research approaches draw upon diverse
4 disciplines including ecology, veterinary science and the social sciences.
5

1 Figure 1

2



3

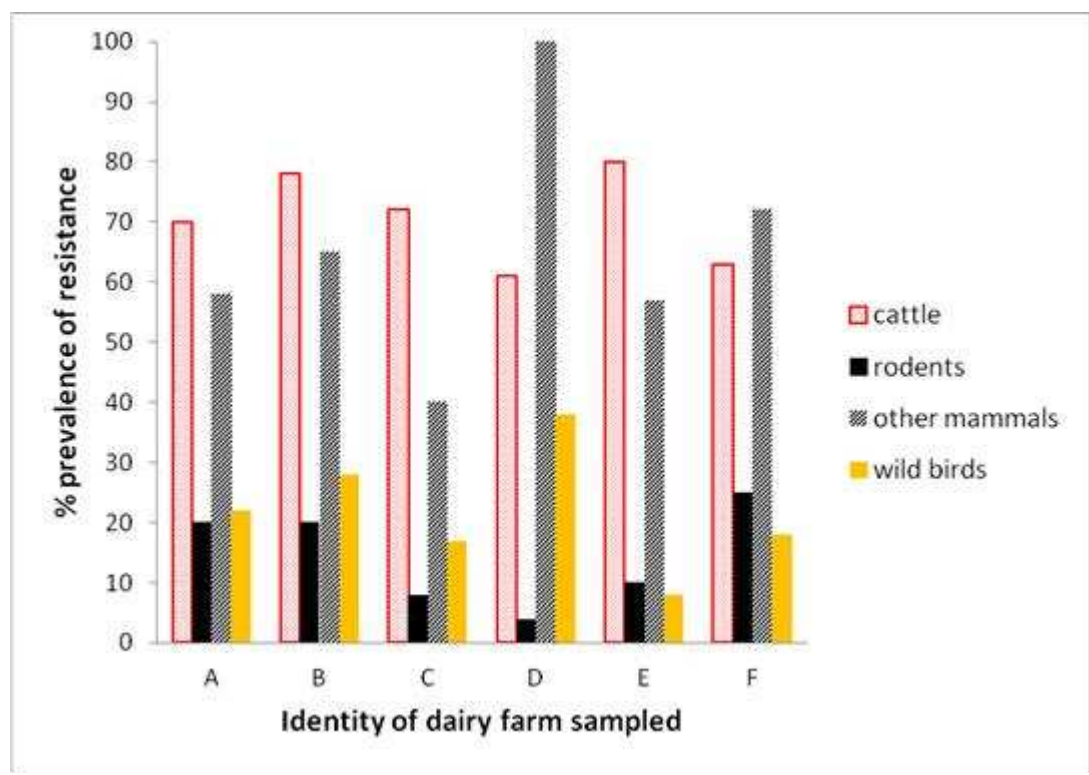
4

5

6

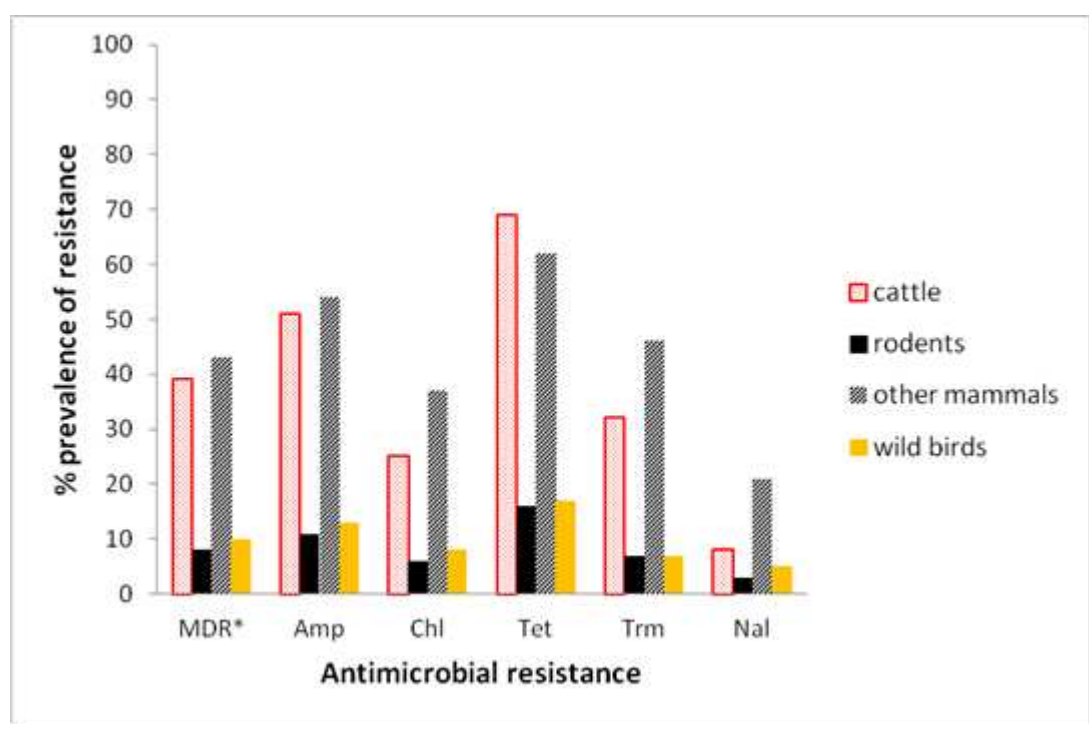
1 Figure 2:

2 A



3

4 B



5

6

1 Table 1

Site type	Predicted exposure to antimicrobial treated livestock	Rodent species sampled ¹	Prevalence (% samples containing resistant <i>E.coli</i>) of antibiotic resistance in faecal samples of different rodent populations					
			amp	apr	chl	tet	trm	nal
Uninhabited island	None	<i>Water vole</i> ²	10-85	0	2-65	5-75	5-85	0-35
Upland forest	Upland sheep	<i>Field vole</i> , <i>Bank vole</i> & <i>Wood mouse</i>	0-5	0	0-5	0-5	0-5	0
Lowland woodland	Reared gamebirds & cattle on adjacent dairy farms	<i>Bank vole</i> & <i>Wood mouse</i>	12-22	0-1	4-6	12-18	11-18	0
Fields on dairy farms	Cattle on fields	<i>Wood mouse</i>	0-27	0	0-27	0-30	0-42	0
		<i>Cattle</i>	5-75	0	0	8-92	5-92	0
Intensive poultry farms	Poultry in buildings	<i>House mouse</i> ³	47-55	0	21-25	57-75	57-68	0-10
		<i>Bank vole</i> & <i>wood mouse</i>	0-5	0	0	0-9	0-12	0
		<i>Poultry</i>	5-8	0	0	15-45	3-6	0-18

2 ¹Rodent species – Water vole *Arvicolis terrestris*, Field vole *Microtus agrestis*; Bank
3 vole *Myodes glareolus*; Wood mouse *Apodemus sylvaticus*; House mouse *Mus*
4 *musculus*. ² Water voles on these islands are fossorial rather than riparian as on the
5 mainland. ³ captured in and around the buildings housing poultry.

6
7

1 Table 2: Summary of some of the key outstanding questions, mitigation measures
 2 and research approaches regarding the role of wildlife in the transmission of AMR
 3 based on the literature reviewed.

Ecology of the host	Biology of the host	Risks to humans and livestock	Mitigation measures	Research approaches
How do species and climate driven differences in seasonal population dynamics affect AMR carriage?	How long and far are resistance genes carried and shed by wildlife (particularly migratory) species?	How can we prove the direction of AMR transmission from humans or livestock to/from wildlife?	How can anthropogenic wastes be managed to prevent transmission of AMR to wildlife?	Spatially explicit field studies & network modelling to identify key transmission locations, species & individuals.
Are carnivores and scavengers more likely to harbour AMR than omnivores or herbivores?	Do gut bacteria endemic to wildlife species differ in their propensity to share resistance genes via horizontal transfer?	Are wild animals a direct (bushmeat) or indirect (contaminating livestock food) route by which AMR can enter the human food chain?	Can existing surveillance and monitoring schemes (e.g. WILDCOMS) be used for AMR screening of wildlife?	Mine AMR metagenome sequences in public databases to test hypotheses regarding ecology & evolution of AMR transmission.
Are group living species or individuals more likely to carry AMR than solitary ones?	Are species with an aquatic life stage or aquatic diet most at risk of acquiring or transmit AMR?	What is the relative contribution of aquaculture to AMR evolution and transmission in aquatic and marine ecosystems?	Do unmetabolised antibiotics from wastes select for the evolution or maintenance of AMR in the environment?	Sensor technology deployed systematically to detect AMR and/or antibiotics in high risk ecosystems, exposure pathways or species.
Are urban adaptors or exploiters more likely to disperse AMR than urban avoider species?	Does an individual's immune function affect its propensity to be infected by AMR microbes?	Which agricultural, religious or cultural practices globally expose humans to wildlife disseminated AMR?	What are the alternatives to culling AMR-infected wildlife that pose a risk to humans?	Deliberative, stake-holder driven, approaches to developing societal solutions to AMR transmission in the environment.

4
5