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- 1 Salmonella Hadar linked to two distinct transmission vehicles highlights challenges to enteric
- 2 disease outbreak investigations
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Non-typhoidal *Salmonella* is a common cause of enteric disease in humans and can be 24 25 transmitted through food and contact with live animals. In 2020, an outbreak of Salmonella 26 Hadar illnesses was linked to contact with non-commercial, privately-owned (backyard) poultry 27 including live chickens, turkeys, and ducks, resulting in 848 illnesses. From late 2020 into 2021, 28 this Salmonella Hadar strain caused an outbreak that was linked to ground turkey consumption. Core genome multilocus sequence typing (cgMLST) analysis determined that the Salmonella 29 Hadar isolates detected during the outbreak linked to backyard poultry and the outbreak linked 30 to ground turkey were closely related genetically (within 0–16 alleles). Epidemiologic and 31 traceback investigations were unable to determine how Salmonella Hadar detected in backyard 32 poultry and ground turkey were linked, despite this genetic relatedness. Enhanced molecular 33 characterization methods, such as analysis of the pangenome of Salmonella isolates, might be 34 necessary to understand the relationship between these two outbreaks. Similarly, enhanced 35 data collection during outbreak investigations and further research could potentially aid in 36 determining if these transmission vehicles are truly linked by a common source and what 37 reservoirs exist across the poultry industries that allow Salmonella Hadar to persist. Further 38 work combining epidemiologic data collection, more detailed traceback information, and 39 genomic analysis tools will be important in the future of monitoring and investigating enteric 40 disease outbreaks. 41

42

43 Introduction

Non-typhoidal Salmonella enterica causes over one million infections in the United States 44 annually [1, 2]. Multistate outbreaks of Salmonella infections occur every year and are linked to 45 food products or contact with animals or their environments [3]. Salmonellosis is a nationally 46 47 notifiable disease in the United States [4]. When Salmonella is isolated by culture from ill people's specimens, state and local public health laboratories perform whole genome 48 sequencing (WGS) on resulting bacterial isolates and upload the data to PulseNet, the national 49 molecular subtyping network for enteric disease surveillance centralized at the United States 50 Centers for Disease Control and Prevention (CDC) [5-7]. PulseNet utilizes core genome 51 multilocus sequence typing (cgMLST) analysis to detect nationwide outbreaks of salmonellosis. 52 53 CDC, along with federal, state, and local public health partners, will initiate outbreak investigations if Salmonella isolates are temporally clustered and cgMLST analysis indicates a 54 high degree of genetic relatedness. Genetically related isolates are more likely to share a 55 common transmission source [5]. Public health officials conduct interviews of ill people to 56 57 identify possible sources of infection and to direct further laboratory testing and traceback of 58 contaminated foods or animal reservoirs. Investigation of outbreaks of genetically related isolates might identify a discrete source of contamination to target interventions for preventing 59 illnesses, but investigations might also fail to identify a source, or might reveal that a strain is 60 61 widely disseminated across a specific industry [8, 9].

Non-commercial, privately owned (also referred to as "backyard") poultry, such as
chickens, turkeys, and ducks, are an increasingly common source of zoonotic transmission of *Salmonella* because of their growing popularity in the United States [10]. Poultry can harbor

Salmonella in their gastrointestinal tract that can be intermittently shed in excreta and 65 66 transmitted to humans, even while the animal appears healthy. Backyard poultry contact is 67 commonly associated with sporadic human Salmonella illness, and multistate outbreaks linked to backyard poultry occur annually, coinciding with the increased sale and distribution of 68 69 backyard poultry across state lines in the spring each year. There are approximately 20 mailorder hatcheries throughout the United States that contribute most of the backvard poultry to 70 U.S. consumers, either directly to consumers from the hatchery or indirectly to consumers 71 through hatcheries partnering with one another and sharing distribution or by supplying 72 agricultural feed stores [11, 12]. Poultry sourcing and distribution practices among mail-order 73 hatcheries have been described [13]. Investigations of backyard poultry-associated Salmonella 74 outbreaks have identified specific sources of contamination along the distribution chain [14], 75 but these outbreak strains might also be widely disseminated among backyard poultry 76 hatcheries and retailers [12]. This growing problem necessitates public health intervention 77 through owner education as well as industry-level pathogen mitigation efforts [13]. 78

Consumption of contaminated poultry products is a major contributor to the overall 79 burden of Salmonella infections and can result in Salmonella illness outbreaks [15]. Historically, 80 outbreaks of foodborne Salmonella Hadar infections were most commonly associated with 81 82 retail turkey products [16]. Turkeys raised to be slaughtered and processed for food are 83 produced through systems that are generally distinct from those that provide animals to the backyard poultry market. Poultry raised for the commercial food industry are usually not sold 84 85 live to members of the public. Individuals wishing to obtain backyard poultry may buy through 86 agricultural feed stores that are supplied by hatcheries, mail order direct from hatcheries, or

private farms or flea markets [13]. Therefore, multistate Salmonella Hadar outbreaks where a 87 88 closely related genetically outbreak strain has been attributed to both backyard poultry and poultry food products have not been previously reported, to our knowledge. However, 89 implementation of WGS has improved our ability to detect *Salmonella* in different products. 90 In 2020, backyard poultry were implicated as the cause of a multistate outbreak of 91 Salmonella Hadar infections. Later that year and into 2021, CDC, along with federal and state 92 partners, investigated another multistate outbreak of Salmonella Hadar and identified ground 93 turkey as the source of illness [17]. Salmonella Hadar isolates obtained from both outbreaks 94 95 were highly related based on cgMLST analysis. This study compares the investigations and 96 findings behind each outbreak and examines explanations provided by epidemiologic and advanced genomic analyses underlying the phenomenon of two outbreaks with exposures to 97 distinct vehicles resulting from a closely related genetically Salmonella Hadar strain. 98 Methods 99

100 The reported outbreak investigation activities were reviewed by CDC and were 101 conducted consistent with applicable federal law and CDC and the U.S. Department of 102 Agriculture, Food Safety and Inspection Service (FSIS) policy.[§]

103 Backyard Poultry-Associated Outbreak

In April 2020, PulseNet notified CDC epidemiologists of 15 ill people from 11 states
 infected with *Salmonella* Hadar that was genetically related within 0–7 allele differences by

[§] See e.g., 45 C.F.R. part 46, 21 C.F.R. part 56; 42 U.S.C. §241(d); 5 U.S.C. §552a; 44 U.S.C. §3501 et seq., 21 U.S.C. 451, et seq

cgMLST analysis. Preliminary data available on patient exposures through routine state or local 106 107 health department interviews indicated nine of ten ill people with available information reported contact with backyard poultry. State and local public health officials continued to 108 109 collect and share patient exposures (including foods eaten and animals contacted, among other 110 general exposures) identified through routine state or local health department interviews throughout the duration of the investigation. Public health officials conducted additional 111 patient interviews whenever possible with a supplemental standardized questionnaire 112 113 examining types of poultry exposure and poultry purchase locations such as feedstores, local farms, and agricultural co-ops. III people were asked about their poultry purchasing since 1 114 January 2020, thus, allowing investigators to better identify traceable records from purchase 115 location to source hatchery. During interviews, ill people were asked if they were willing to 116 have their backyard flocks sampled for *Salmonella*. Questionnaire responses were collected in 117 CDC's Epi Info[™] Web Survey and aggregated using the System for Enteric Disease Response, 118 Investigation, and Coordination (SEDRIC) [18, 19]. A case was ultimately defined as Salmonella 119 Hadar infection yielding an isolate, related within 0–15 allele differences based on cgMLST, 120 from a patient with illness onset dates from 26 February 2020, through 11 November 2020 [9, 121 20, 21]. Patient response data were analyzed using SAS software version 9.4 (Cary, NC, USA). All 122 123 clinical isolates have been deposited to the National Center for Biotechnology Information 124 (NCBI) BioProject PRJNA230403.

125 State and local public health and agricultural officials in Kentucky, New Hampshire, and 126 Oregon conducted sampling of backyard poultry and their environments at ill people's homes 127 using standard procedures [22]. These samples were processed by their respective public health laboratories utilizing standardized aerobic culture methods [23] and PulseNet WGS
protocols [20]. WGS data were uploaded to the PulseNet national database and compared to
outbreak patient sequences.

CDC epidemiologists utilized information from patient interviews to identify any 131 backyard poultry hatcheries or suppliers that could have been a common source of backyard 132 poultry resulting in transmission of Salmonella Hadar in this outbreak. Some ill people reported 133 how and where they acquired their poultry, many of whom had purchased from agriculture 134 feedstores. Some feedstore locations were part of corporations; CDC shared purchase 135 136 information for purchases since 1 January 2020 with feedstore corporations (>100 store 137 locations) to identify the hatcheries that supplied poultry to their stores. Employees of 138 independent feedstores, farms, agriculture co-ops, and small feedstore corporations (<100 store locations) where ill people had purchased poultry were interviewed with a standardized 139 140 questionnaire regarding poultry breeds and species sold and source hatcheries based on ill people's reported purchase dates. 141

142 Ground Turkey-Associated Outbreak

In February 2021, PulseNet notified CDC epidemiologists of 17 cases of *Salmonella*Hadar infection with specimen collection dates since January 1, 2021, that were related within
10 allele differences by cgMLST analysis. These isolates were also genetically related to the
2020 backyard poultry-associated outbreak. Because of genetic similarities between patient
isolates, ground turkey isolates, and isolates from the 2020 backyard poultry-associated
outbreak in the PulseNet database, state and local health officials collected information on the

types of poultry products consumed in the seven days before illness onset, including brand and packaging information and location of purchase, as well as exposures to backyard poultry. Ill people were asked if they had food product available for *Salmonella* testing. Questionnaire responses were aggregated using SEDRIC. A case was defined as *Salmonella* Hadar infection yielding an isolate, related within 0-8 allele differences based on cgMLST, from a patient with illness onset dates occurring 28 December 2020, to 22 April 2021 [9, 20, 24].

During the course of the outbreak investigation, FSIS tested one unopened ground 155 turkey sample collected from a patient's home. This sample was processed utilizing 156 157 standardized FSIS Salmonella culture and WGS protocols [25, 26]. FSIS carries out routine 158 testing of turkey product and cecal samples for enteric pathogens such as Salmonella as part of 159 ongoing surveillance throughout the year either via standard food safety monitoring dictated by federal directive [27] or as part of the National Antimicrobial Resistance Monitoring System 160 161 (NARMS) [28]. The U.S. Food and Drug Administration (FDA) oversees Salmonella testing of 162 ground turkey purchased from retail establishments through the NARMS program [29]. Sampling, culture methods, and WGS of Salmonella isolates performed by FSIS and FDA follow 163 standard protocols described elsewhere [25, 26, 30]. WGS data of these isolates are routinely 164 uploaded to PulseNet. 165

FSIS obtained information for *Salmonella*-positive retail ground turkey samples to determine where the products were processed. FSIS also worked with public health partners to obtain patient product purchase records from information reported in patient interviews (*i.e.*, retail store shopper card numbers) to determine if there was a common processing establishment or brand associated with patient illness.

171 Results

172 Backyard Poultry-Associated Outbreak

173 The investigation identified 848 people ill with the outbreak strain in 49 states (Figure 174 1a). Illness onset dates ranged from 26 February 2020, through 11 November 2020 (Figure 2). Ages ranged from <1 to 95 years with a median of 36 years, and 216 of 840 (26%) were children 175 under the age of 5 years; 480 of 811 (59%) were female. Of ill people with available 176 information, 186 of 542 (34%) were hospitalized, and there were no reported deaths. Of 476 ill 177 people with animal exposure information available from either routine or supplemental 178 interview, 346 (73%) reported contact with backyard poultry. Among 159 ill people who 179 provided information about the types of poultry they had contact with, most reported contact 180 with chickens (70%, n=112) or ducks (43%, n=69). Ill people also reported contact with other 181 poultry including turkeys (5%, n=8), geese (3%, n=5), or guineas (3%, n=5). These patients 182 primarily described the poultry they contacted as "baby" poultry (76%, n=121), while some had 183 contact with "adult" poultry (30%, n=49). Ill people were also queried about the breeds or types 184 of chickens or ducks that they contacted; 109 ill people provided the breed or type of chicken 185 contacted, and 33 ill people provided the breed or type of duck contacted. Twenty-eight 186 different breeds of chickens and 15 breeds of ducks were reported. Among 438 ill people with 187 188 routine interview data shared, 25 ill people reported turkey consumption of various types (i.e., ground, deli/sliced), 8 of which also had contact with backyard poultry prior to their illness 189 190 onset.

191 Testing of poultry and their environment yielded six *Salmonella* Hadar isolates: four 192 isolates were collected from three duck cloacae and their environment at a patient's home in 193 Kentucky, one was obtained from another duck pen area at an ill patient's home in Oregon, and 194 one was obtained from a chicken's excreta at a patient's home in New Hampshire. All six 195 isolates were highly related to each other and the corresponding patient isolates within 0–4 196 allele differences. No poultry feed samples were tested.

Among 346 ill people with backyard poultry contact, 210 (61%) reported purchasing 197 poultry since 1 January 2020. Two hundred and ten ill people reported a total of 223 distinct 198 199 purchases from at least 48 companies, including mail-order hatcheries, corporate and independent farms, or feedstores, from 155 unique locations. Eight questionnaires 200 administered to storefronts across five independently owned and operated companies were 201 returned detailing where they sourced poultry. Additionally, source hatcheries were identifiable 202 203 for 26 store locations included as part of two large corporations. In total, 34 (22%) purchase locations belonging to seven companies provided source hatchery information. These store 204 locations traced to 10 different hatcheries located in 8 states (Figure 3). For hatcheries 205 identified in traceback, information could not be obtained regarding the sources of poultry 206 among these hatcheries or whether these hatcheries shared any common suppliers. 207

208 Ground Turkey-Associated Outbreak

This investigation identified 34 people ill with the outbreak strain from 15 states (Figure 1b). Illness onset dates occurred from 28 December 2020, through 22 April 2021 (Figure 2). Ages ranged from <1 to 92 years, with a median of 49 years, and 21 (62%) ill people were

female. Four (18%) of 22 with available information were hospitalized, and no deaths were 212 213 reported. Thirteen ill people responded to requests for interview with the questionnaire. Eight (62%) of 13 ill people who were asked specifically about turkey exposures reported eating 214 215 ground turkey within seven days of becoming ill. This was significantly higher than the 13% of 216 healthy people who reported eating ground turkey the week prior to interview in the 2018-2019 FoodNet Population survey (p<0.001) [31]. An additional two people reported eating 217 turkey products other than ground turkey within seven days of becoming ill. Ill people reported 218 purchasing seven different brands of turkey products. No ill people reported owning or 219 contacting backyard poultry directly. One patient reported eating chicken and duck eggs 220 provided from their neighbor. 221

Twenty-nine isolates of the outbreak strain were obtained from turkey samples from 14 222 slaughter or processing establishments: 12 isolates detected through FSIS regulatory sampling 223 224 of ground turkey at production facilities, three isolates obtained through FSIS NARMS sampling of turkey ceca, seven isolates identified through NARMS surveillance efforts by FDA and state 225 partners of retail ground turkey products, and seven isolates from an unopened package of 226 ground turkey at an ill patient's home in Pennsylvania. Six of the seven isolates from the 227 product at the Pennsylvania home were indistinguishable (0 allele differences) from the isolate 228 229 collected from the patient. Isolates from ill people, turkey cecal contents, and ground turkey 230 products were related within 0–8 allele differences by cgMLST. The ground turkey sampled from the ill patient's home also yielded six isolates of Salmonella serotype I 3,10:e,h:-, which 231 232 was not isolated from any ill people or genetically related to the outbreak strain. One isolate of 233 genetically related Salmonella Hadar from a chicken product was reported through FSIS

regulatory sampling. This chicken product sample was obtained from an establishment that
 processes both chicken and turkey products.

236 FSIS conducted traceback of ground turkey purchases for six ill people from four states. No single retail store or processing establishment could be linked to all ill people. Multiple 237 suppliers were identified during traceback; two establishments ("Establishments X and Y") were 238 the sole supplier of ground turkey purchased by two ill people each. Two ill people (one from 239 Maryland and one from Maine) ate ground turkey product that was traced to Establishment X; 240 two ill people from Pennsylvania ate ground turkey that traced back to Establishment Y; and 241 242 two ill people (one from Pennsylvania who allowed testing of ground turkey remaining in their 243 home and one from Connecticut) ate ground turkey traced back to multiple suppliers, including both Establishments X and Y. Establishments X and Y were among 14 establishments located in 244 11 states that had turkey isolates included in the investigation. 245

Isolates from ill people included in the ground turkey-associated outbreak were closely
genetically related within 0–16 alleles by cgMLST to isolates included in the backyard poultryassociated outbreak (Figure 4). No backyard poultry were sampled during the ground turkeyassociated outbreak because no ill people reported backyard poultry contact or ownership.

250 Discussion

We report two multistate outbreaks linked to distinct vehicles but caused by *Salmonella* Hadar that was closely genetically related as determined by cgMLST (within 0–16 alleles). The emergence of this strain in 2020, the high number of illnesses that resulted, the persistence of transmission, and the dissemination in backyard poultry and food poultry industries are of

public health concern. Backyard poultry-associated Salmonella Hadar illnesses contributed to 255 256 an overall 617% (95% CI: 382–987%) increase in Salmonella Hadar in 2020 compared to 2017– 2019 [32]. Additionally, Salmonella Hadar is one of the most common serotypes isolated from 257 food-producing turkeys and derived products in North America [33-35]. Turkey products have 258 259 contributed to both single and multistate outbreaks of Salmonella Hadar in the United States [36], but it has not been previously established that these outbreaks are genetically, 260 epidemiologically, or otherwise related to Salmonella Hadar strains transmitted to people from 261 262 backyard poultry.

263 The two outbreaks reported here were investigated as two distinct events and the 264 epidemiologic, laboratory, and traceback evidence collected during these investigations have yet to explain how these outbreaks, linked to distinct vehicles, resulted from a closely 265 genetically related Salmonella Hadar strain (within 0–16 alleles by cgMLST). During the 266 267 backyard poultry-associated outbreak, ill people might have been asked about food exposures 268 through routine state or local health department interviews, but these questions are not standardized across jurisdictions; exposure to turkey products was reported by ill people but 269 was infrequent, with a small number of ill people reporting backyard poultry exposure and 270 turkey consumption. Of note, not all ill people in the backyard poultry-associated outbreak 271 272 were asked about turkey food product exposure, and reporting might have been subject strictly 273 to patient recall when asked about general food exposures in the week prior to illness onset. This could have artificially reduced the number of ill people in this outbreak reporting ground 274 275 turkey exposure. Furthermore, routine sampling of turkey by FSIS, FDA, and state and local 276 public health officials was ongoing throughout the backyard poultry-associated outbreak [27277 29]. The outbreak strain was detected in ground turkey during the backyard poultry-associated 278 outbreak investigation, but because of the increased number of ill people reporting backyard 279 poultry contact during that time, additional follow-up of ground turkey consumed by patients was not conducted as part of the backyard poultry-associated outbreak investigation. 280 281 Systematically questioning patients about food poultry exposures during this investigation could have revealed that some people were becoming ill as a result of ground turkey at the 282 same time that people were known to be exposed to Salmonella Hadar via contact with 283 backyard poultry, and this could have identified additional measures to prevent illnesses during 284 this outbreak. During the ground turkey-associated outbreak, ill people were specifically asked 285 about exposure to backyard poultry, and none reported direct contact or ownership. 286 In both outbreaks, some ill people could not be interviewed, and no exposure 287 information was available from them, as is typical for enteric disease outbreak investigations. 288 Therefore, it is possible that ill people in either outbreak were exposed to the outbreak strain 289 290 by a different vehicle. These Salmonella Hadar outbreaks illustrate the importance of collecting detailed epidemiologic evidence to characterize food and animal exposures. When further 291 outbreaks of this Salmonella Hadar strain occurred after 2021, investigators questioned ill 292 people in detail about their exposure to food turkey products and backyard poultry, and this 293 294 has aided in determining which ill people have been exposed by contaminated foods and which 295 by animal contact.

cgMLST analysis demonstrates that food, animal, and clinical isolates from both
 outbreaks were closely genetically related (within 0–16 alleles). In 2019, WGS became the
 standard molecular subtyping approach for foodborne disease surveillance across PulseNet

participating public health laboratories; this replaced the previous method of pulsed-field gel 299 300 electrophoresis (PFGE) and introduced substantially higher precision when identifying ill people during outbreak investigations [5, 37]. This was particularly useful in distinguishing isolates of 301 302 clonal *Salmonella* serotypes that demonstrate minimal genetic variation over time and were 303 indistinguishable by PFGE [5]. Salmonella Hadar demonstrates such clonality; of 3047 isolates of Salmonella Hadar available in the PulseNet database as of July 2023, 2143 (70%) are related 304 within 0-26 alleles by cgMLST [38]. cgMLST compares genes identified in >97% of the strains of 305 a given bacterial species, which, in *Salmonella*, consists of 3002 loci [24, 39]; however, this does 306 not examine the accessory genome of isolates, which is a collection of highly variable genes 307 that might be shared between bacteria via horizontal transfer as plasmids, transposable 308 elements, or other mobile genetic material [40]. Different methods might be employed to 309 analyze WGS data that provide varying degrees of granularity in evaluating the genetic 310 relatedness between strains. Analysis of the complete Salmonella Hadar pangenome might 311 allow distinction between source exposures in future ill people infected with this strain, and at 312 the time of writing, the authors are investigating the utility and limitations of such an analytic 313 approach for describing Salmonella Hadar. 314

In addition to epidemiologic and laboratory evidence, traceback investigations conducted during both outbreaks were not able to explain how backyard poultry could be linked to or transmit *Salmonella* Hadar that some people later acquired from exposure to or consumption of contaminated ground turkey. Ill people in the backyard poultry-associated outbreak primarily reported contact with chickens and ducks, and live turkey contact was reported infrequently. It is unknown how frequently poultry sold for backyard keeping overlap

during their life cycle with those raised and processed for commercial food production. In some 321 322 instances, commercial poultry egg suppliers do supply hatching eggs or live young birds to 323 backyard poultry hatcheries that subsequently supply agricultural feed stores [14]. However, 324 further information needs to be collected from industry partners to fully understand if there is 325 a plausible connection in the poultry supply chain linking commercial food producers and backyard poultry hatcheries. One hypothesis which might explain the finding of the strain in 326 different sectors of the poultry industry is that backyard poultry and commercially produced 327 turkeys associated with each outbreak received the same feed that was contaminated with the 328 implicated Salmonella Hadar strain. Contaminated animal feed is a documented source of 329 Salmonella outbreaks in people [41]. Patient interviews did not identify a common feed 330 administered between backyard poultry owners, nor were feed samples tested during the 331 investigation. Additionally, while traceback of ground turkey product samples and ground 332 turkey purchased by ill people identified processing establishments for some products, the 333 investigation did not identify farms at which turkeys were raised before processing, thus 334 precluding on-farm follow-up to examine potential sources of Salmonella Hadar, such as feed, 335 during the outbreak. Salmonella Hadar has historically been one of the most common serotypes 336 isolated from poultry feed in European studies [42, 43], but Salmonella is now reported in less 337 338 than 0.5% of samples taken from poultry feed in the European Union [44]. The FDA Center for 339 Veterinary Medicine monitors for the presence of Salmonella in livestock and poultry feeds and has reported a declining prevalence of Salmonella over time, though Salmonella prevalence in 340 341 feed from the United States is reportedly higher compared to the prevalence in Europe [45]. 342 These efforts, as well as other surveillance studies, have detected Salmonella Hadar in poultry

feed infrequently [45, 46], and some have not detected *Salmonella* Hadar at all [47]. Ultimately,
although feed is a potential commonality between backyard poultry and food production
industries, there is not sufficient evidence to determine if it was a source of *Salmonella* Hadar in
these outbreaks. In the event of future outbreaks of *Salmonella* Hadar, investigators should
consider testing feed samples for *Salmonella* contamination as a means of examining this
hypothesis further.

Since these outbreak investigations, Salmonella Hadar has continued to cause illnesses 349 in people, and additional multistate outbreak investigations have sought to characterize how 350 351 these illnesses might have occurred [48]. Public health officials in the United States are 352 continuing to identify, describe, and track strains of enteric bacteria like Salmonella Hadar that persistently cause illnesses over time despite investigation and prevention efforts [49]. These 353 strains can be detected over wide geographic areas, potentially among large populations of 354 355 animals or in environmental niches, and therefore the approach to respond to and mitigate 356 further transmission of these strains to people requires actions unique from those utilized in acute outbreaks where there is a discrete source of contamination to target interventions [49]. 357 While focal investigations of highly related isolates remain critical to understanding sources of 358 these strains, for persisting strains it is important to leverage collaboration among 359 360 governmental agencies, food and animal industries, and academia to further describe where 361 and how these strains persist—including identifying what reservoirs could be contributing to 362 their spread—and implementing strategies to reduce spread when possible. Complete 363 elimination of these widespread persisting strains is challenging and requires time, sufficient 364 resources, and active engagement across sectors.

365	This report highlights limitations to the standard epidemiologic, laboratory, and
366	traceback methods used by public health agencies to investigate Salmonella strains which
367	might be widely disseminated and result in outbreaks linked to distinct transmission vehicles.
368	Advances in genetic characterization of enteric pathogens like Salmonella have considerably
369	enhanced the ability of disease investigators to respond quickly and effectively to outbreaks.
370	However, in a complex and everchanging globalized food system that is complicated by direct
371	interaction with animals, new approaches and advanced technology are needed to mitigate
372	novel threats and identify circumstances in which individual strains of enteric pathogens could
373	be spread by different vehicles at once. This Salmonella Hadar strain has continued to be
374	associated with backyard poultry and ground turkey [50, 51], and public health officials have
375	bolstered efforts to collect robust epidemiologic information and are actively utilizing advanced
376	molecular characterization techniques to learn more about this strain.
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- 387 **Data availability statement:** The data that support the findings of this study are available from
- the authors upon reasonable request. All clinical isolates have been deposited to the National
- 389 Center for Biotechnology Information (NCBI) BioProject PRJNA230403.

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390 References

(1)

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392 Emerging Infectious Diseases; 17: 7-15. 393 CDC. (2019) Antibiotic Resistance Threats in the United States. U.S. Department of Health and (2) 394 Human Services, CDC. Atlanta, GA. 395 Hale CR, et al. (2012) Estimates of enteric illness attributable to contact with animals and their (3) 396 environments in the United States. Clinical Infectious Diseases; 54 Suppl 5: S472-479. 397 (4) CDC. National Notifiable Disease Surveillance System (NNDSS) Salmonellosis (Salmonella spp.). 398 (2021) In. Accessed from: https://ndc.services.cdc.gov/conditions/salmonellosis/. 399 (5) Kubota KA, et al. (2019) PulseNet and the changing paradigm of laboratory-based surveillance 400 for foodborne diseases. Public Health Report; 134: 22S-28S. 401 (6) McDermott PF, et al. (2016) Whole-Genome Sequencing for Detecting Antimicrobial Resistance 402 in Nontyphoidal Salmonella. Antimicrobial Agents and Chemotherapy; 60: 5515-5520. 403 (7) Tolar B, et al. (2019) An overview of PulseNet USA Databases. Foodborne Pathogens and 404 Disease; 16: 457-462. 405 (8) FSIS. Salmonella Reading illness outbreak associated with turkey, 2017-2019. (2020) In. 406 Accessed from: https://www.fsis.usda.gov/sites/default/files/media_file/2020-407 11/Salmonella%20Reading%20Illness%20Outbreak%20Associated%20with%20Turkey%2C%202017%E2 %80%9<u>32019.pdf</u>. 408 409 Marshall KE, et al. (2020) Investigations of Possible Multistate Outbreaks of Salmonella, Shiga (9) 410 Toxin-Producing Escherichia coli, and Listeria monocytogenes Infections - United States, 2016. MMWR 411 Surveillance Summary; 69: 1-14. 412 Pires AFA, et al. (2020) Assessment of veterinarians' engagement with backyard poultry and (10)413 small-scale livestock operations in four western states. Journal of the American Veterinary Medical 414 Association; 257: 196-209. 415 (11)Basler C, et al. (2016) Outbreaks of Human Salmonella Infections Associated with Live Poultry, 416 United States, 1990-2014. Emerging Infectious Diseases; 22: 1705-1711. 417 Behravesh CB, et al. (2014) Backyard poultry flocks and salmonellosis: a recurring, yet (12)418 preventable public health challenge. Clinical Infectious Diseases; 58: 1432-1438. 419 (13)Nichols M, et al. (2018) Preventing Human Salmonella Infections Resulting from Live Poultry 420 Contact through Interventions at Retail Stores. Journal of Agricultural Safety and Health; 24: 155-166. 421 (14)Robertson SA, et al. (2019) Onsite investigation at a mail-order hatchery following a multistate 422 Salmonella illness outbreak linked to live poultry-United States, 2018. Poultry Science; 98: 6964-6972. 423 (15)Antunes P, et al. (2016) Salmonellosis: the role of poultry meat. Clinical Microbiology and 424 Infection; 22: 110-121. 425 (16)Jackson B, et al. (2013) Outbreak-associated Salmonella enterica Serotypes and Food 426 Commodities, United States, 1998–2008. Emerging Infectious Disease; 19: 1239. 427 (17) CDC. Salmonella outbreak linked to ground turkey. (2021) In: U.S. Department of Health and 428 Human Services C, ed. Accessed from: https://www.cdc.gov/salmonella/hadar-04-21/details.html. 429 (18) CDC. Epi Info. (2021) In. Accessed from: https://www.cdc.gov/epiinfo/index.html. 430 CDC. SEDRIC Overview. (2020) In. Accessed from: (19) 431 https://www.cdc.gov/foodsafety/outbreaks/investigating-outbreaks/sedric/sedric-overview.html. 432 (20) CDC. PulseNet methods and protocols: Whole genome sequencing (WGS). (2016) In. Accessed 433 from: https://www.cdc.gov/pulsenet/pathogens/wgs.html. 434 (21) Gerner-Smidt P, et al. (2019) Whole Genome Sequencing: Bridging One-Health Surveillance of 435 Foodborne Diseases. *Frontiers in Public Health*; **7**: 172.

Scallan E, et al. (2011) Foodborne illness acquired in the United States--major pathogens.

436 (22)USDA. Code of Federal Regulations Title 9 - Animal and Animal Products Chapter 1 § 147.12 437 Procedures for collection, isolation, and identification of Salmonella from environmental 438 samples, cloacal swabs, chick box papers, and meconium samples. (2021) In: Agriculture USDo, ed. 439 Washington, D.C. Accessed from: https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/veterinary-440 biologics/biologics-regulations-and-guidance/CT Vb cfr. 441 Andrews WH, et al. Bacteriological Analytical Manual. In. 8th Edition ed: United States Food and (23) 442 Drug Administration, 2021. 443 (24)Besser JM, et al. (2019) Interpretation of whole-genome sequencing for enteric disease 444 surveillance and outbreak investigation. Foodborne Pathogens and Disease; 16: 504-512. 445 (25) FSIS. FSIS Microbiology Laboratory Guidebook: Isolation and identification of Salmonella from 446 meat, poultry, pasteurized eggs, and siluriformes (fish) products and carcass and environmental 447 sponges. (2022) In. Accessed from: https://www.fsis.usda.gov/sites/default/files/media_file/documents/MLG-4.12.pdf. 448 FSIS. FSIS Microbiology Laboratory Guidebook: Whole genome sequencing of bacterial isolates. 449 (26) 450 (2020) In. Accessed from: https://www.fsis.usda.gov/sites/default/files/media_file/2021-03/mlg-42.pdf. 451 FSIS. FSIS Microbiology Laboratory Guidebook: Salmonella and Campylobacter verification (27) 452 program for raw poultry products. (2021) In. Accessed from: 453 https://www.fsis.usda.gov/sites/default/files/media file/2021-03/10250.1.pdf. 454 (28) FSIS. FSIS cecal sampling under the National Antimicrobial Resistance Monitoring System 455 (NARMS) surveillance program - revision 2. FSIS Directive 10100.1. (2022) In. Accessed from: 456 https://www.fsis.usda.gov/policy/fsis-directives/10100.1. FDA. The National Antimicrobial Resistance Monitoring System: About NARMS. (2020) In. 457 (29) 458 Accessed from: https://www.fda.gov/animal-veterinary/national-antimicrobial-resistance-monitoring-459 system/about-narms. 460 (30) NARMS. The National Antimicrobial Resistance Monitoring System: Manual of Laboratory 461 Methods. (2021) In. Accessed from: https://www.fda.gov/media/101423/download. CDC. FoodNet Fast Population Survey Tool. (2021) In. Atlanta, GA: Centers for Disease Control 462 (31) 463 and Prevention Foodborne Diseases Active Surveillance Network. Accessed from: 464 https://wwwn.cdc.gov/Foodnetfast/PopSurvey. 465 (32) Ray LC, et al. (2021) Decreased Incidence of Infections Caused by Pathogens Transmitted Commonly Through Food During the COVID-19 Pandemic - Foodborne Diseases Active Surveillance 466 467 Network, 10 U.S. Sites, 2017-2020. MMWR Morbidity and Mortality Weekly Report; 70: 1332-1336. 468 (33) Anderson PN, et al. (2010) Molecular analysis of Salmonella serotypes at different stages of 469 commercial turkey processing. Poultry Science; 89: 2030-2037. 470 (34) Caffrey N, et al. (2021) Salmonella spp. prevalence and antimicrobial resistance in broiler 471 chicken and turkey flocks in Canada from 2013 to 2018. Zoonoses and Public Health. 472 Morningstar-Shaw B, et al. Salmonella Serotypes Isolated from Animals and Related Sources. (35) 473 (2016) In: NVSL U, ed. Ames, IA. Accessed from: 474 https://www.cdc.gov/nationalsurveillance/pdfs/salmonella-serotypes-isolated-animals-and-related-475 sources-508.pdf. 476 Jackson BR, et al. (2013) Outbreak-associated Salmonella enterica serotypes and food (36) 477 Commodities, United States, 1998-2008. Emerging Infectious Diseases; 19: 1239-1244. 478 den Bakker HC, et al. (2014) Rapid whole-genome sequencing for surveillance of Salmonella (37) 479 enterica serovar enteritidis. *Emerging Infectious Diseases*; **20**: 1306-1314. 480 PulseNet USA. PulseNet Salmonella National Database, a BioNumerics database v 7.6 used for (38) 481 comparing isolate sequences for surveillance. Part of the standard PulseNet workflow. (2023) In. 482 (39) Alikhan NF, et al. (2018) A genomic overview of the population structure of Salmonella. PLoS 483 Genetics; 14: e1007261.

484 (40)Jacobsen A, et al. (2011) The Salmonella enterica pan-genome. *Microbial Ecology*; 62: 487-504. 485 (41) Crump JA, Griffin PM, Angulo FJ. (2002) Bacterial Contamination of Animal Feed and Its 486 Relationship to Human Foodborne Illness. Clinical Infectious Diseases; 35: 859-865. 487 (42) Vanderwal P. (1979) Salmonella Control of Feedstuffs by Pelleting or Acid Treatment. World's 488 Poultry Science Journal; 35: 70-78. 489 (43) Veldman A, et al. (1995) A survey of the incidence of Salmonella species and Enterobacteriaceae 490 in poultry feeds and feed components. *Veterinary Record*; **136**: 169-172. 491 (44) EFSA. (2021) The European Union One Health 2020 Report. EFSA Journal; 19. 492 (45) Li X, et al. (2012) Surveillance of Salmonella Prevalence in Animal Feeds and Characterization of 493 the Salmonella Isolates by Serotyping and Antimicrobial Susceptibility. Foodborne Pathogens and 494 Disease; 9: 692-698. Sargeant JM, et al. (2021) Salmonella in Animal Feeds: A Scoping Review. Front Vet Sci; 8: 495 (46) 496 727495. (47) Shariat NW, et al. (2021) Incidence of Salmonella serovars isolated from commercial animal 497 498 feed mills in the United States and serovar identification using CRISPR analysis. Journal of Applied 499 *Microbiology*; **130**: 2141-2146. CDC. Persistent strain of Salmonella Hadar (REPTDK01) linked to backyard poultry and ground 500 (48) 501 turkey. (2023) In. Accessed from: https://www.cdc.gov/ncezid/dfwed/outbreak-response/rep-502 strains/reptdk01.html. CDC. Reoccurring, emerging, and persisting enteric bacterial strains. (2023) In. Accessed from: 503 (49) 504 https://www.cdc.gov/ncezid/dfwed/outbreak-response/rep-strains.html. 505 (50) **CDC**. Salmonella outbreaks linked to backyard poultry. (2021) In. Accessed from: 506 https://www.cdc.gov/salmonella/backyardpoultry-05-21/index.html. CDC. Salmonella outbreaks linked to backyard poultry. (2022) In. Accessed from: 507 (51) 508 https://www.cdc.gov/salmonella/backyardpoultry-06-22/index.html.

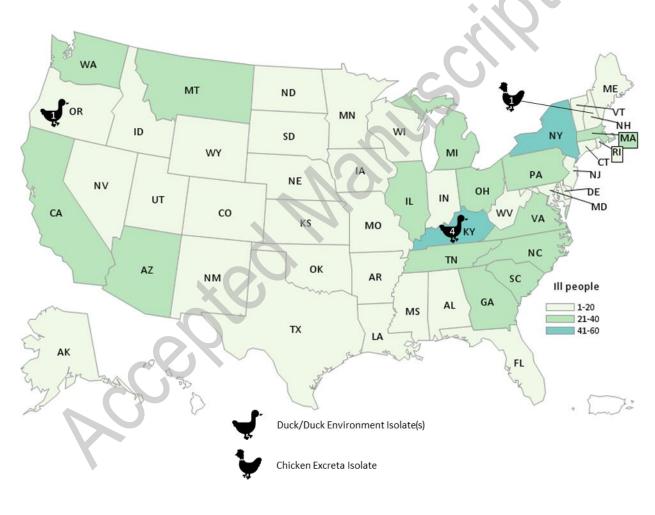
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510 Figure Legends

Figure 1. People infected with the strain of *Salmonella* Hadar by state of residence, identified as
part of the backyard poultry-associated outbreak (a.) and ground turkey-associated outbreak
(b.). Icons and the number within correspond to the number of isolates from that sample type.

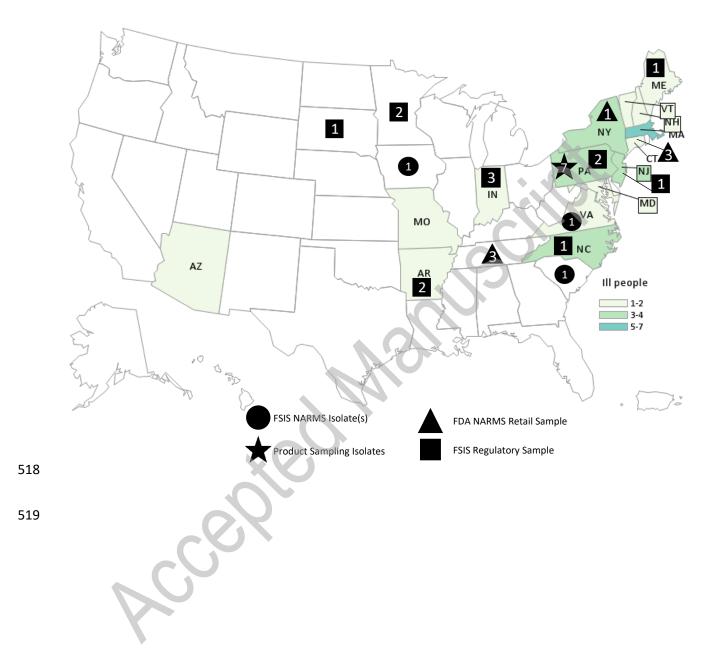
514 **(a.)**



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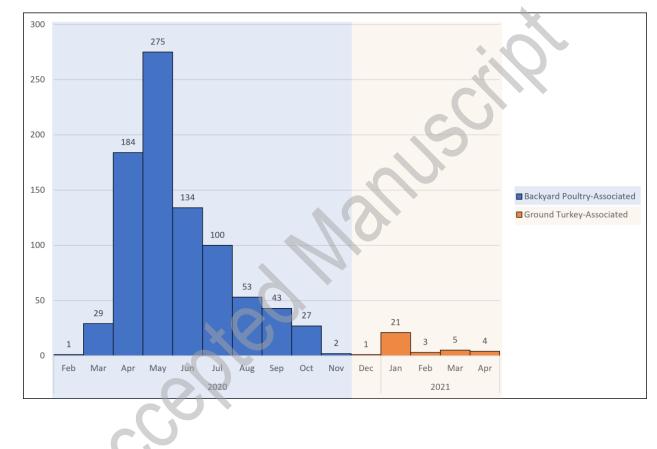
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517 **(b.)**



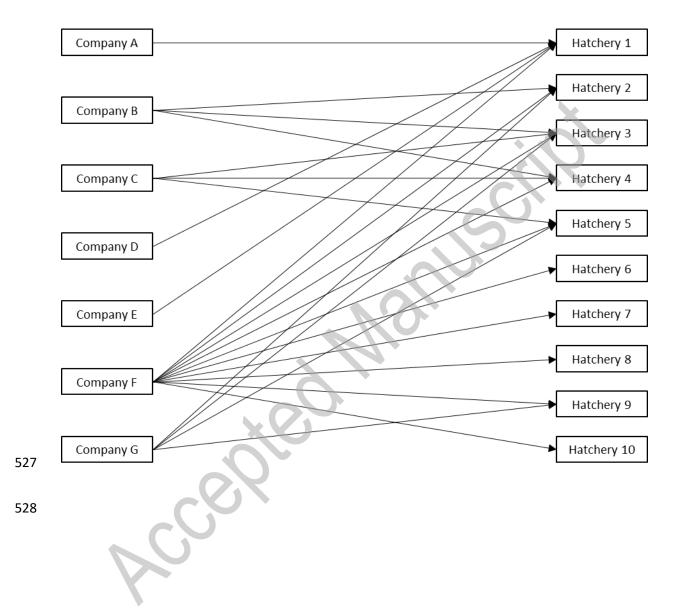
520 **Figure 2.** Epidemic curve of reported illnesses by onset date. People infected with the backyard

- 521 poultry-associated outbreak strain of *Salmonella* Hadar (*n* = 848) and people infected with the
- 522 ground turkey-associated outbreak strain of *Salmonella* Hadar (*n* = 34) by date of illness onset,



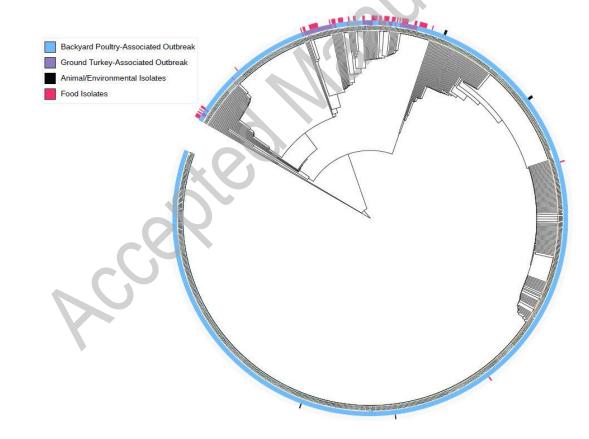
523 United States, 2020–2021.

525 **Figure 3.** Traceback diagram depicting 10 hatchery sources among 7 of 48 (15%) companies



526 with traceable poultry purchase locations in the backyard poultry-associated outbreak.

Figure 4. Core genome multilocus sequence typing analysis of 950 Salmonella Hadar isolates 529 530 related by 0-16 allele differences identified during the backyard poultry-associated outbreak 531 and ground turkey-associated outbreak from human, food, animal, or environmental sources. The inner ring (black color) of this diagram is a phylogenetic tree demonstrating relatedness of 532 the 950 isolates. The middle ring (blue or purple color) designates which outbreak investigation 533 each isolate belongs to. The outermost ring designates if isolates were obtained from food 534 products (pink color) or backyard poultry or their environment (black color); isolates without 535 this label are clinical isolates.



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