

Prevalence and characterization of *Morganella morganii* in beef cattle from Sichuan Province, China

bin Tian

Sichuan Agricultural University

dongjie cai

Sichuan Agricultural University

xiao liu

Sichuan Agricultural University

yulong zhang

Sichuan Agricultural University

jianzhu liu

Shandong Agricultural University

Mingshu Wang

Sichuan Agricultural University

Ya Wang

Sichuan Agricultural University

liping Gou

Sichuan Agricultural University

zhijun zhong

Sichuan Agricultural University

Jing Fang

Sichuan Agricultural University

zhicai zuo (✉ zzcjl@126.com)

Sichuan Agricultural University

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Abstract

Background *Morganella morganii* (*M. morganii*) is a member of the genera *Morganella* in the *Enterobacteriaceae* family.

Methods To better understand the prevalence and characterization of *M. morganii* strains in cattle, 191 nasal swabs samples from beef cattle in Guang'an, Yibin, and Ziyang, Sichuan Province, China were collected. The presence of *M. morganii* in swabs was determined by PCR. Then positive swabs were processed by using bacterial isolation and identification. The *M. morganii* isolates were assayed for antimicrobial susceptibility and pathogenicity using a mouse model.

Results The prevalence of *Morganella morganii* in three cities was 10.5% (20/191). Three *M. morganii* strains (16GA7, 17GA61.2, and 17YB9) were isolated and identified from positive swabs samples. Phylogenetic analysis demonstrated that the 16S rRNA gene of *M. morganii* isolates could be clustered with known Genbank *M. morganii* strains. All three *M. morganii* strains were sensitive to penicillin-type and quinolone antibiotics, and were resistant to some cephalosporins, carbapenems, macrolide antibiotics and polymyxin B. Lesions in mice inoculated with *M. morganii* included pulmonary hemorrhage, thickened alveolar walls, and pulmonary inflammatory cell infiltration, although lesions varied by strain.

Conclusion To the best of our knowledge, the present study is the first to report the prevalence of *M. morganii* in beef cattle. Through research on the *M. morganii* in beef cattle, it is found that mice were susceptible to *M. morganii* isolates, which can cause mice dead. However, the pathogenicity of *M. morganii* isolates on cattle still needs further study.

Background

Morganella morganii (*M. morganii*) is a Gram-negative bacterium in the family *Enterobacteriaceae* that was first isolated in 1906 from a pediatric fecal culture [1]. This bacterium is part of the normal bacterial flora of humans and other animals, and is widely distributed in the environment. *M. morganii* is an opportunistic pathogen that rarely causes infections, but can be associated with a diverse number of conditions, including sepsis, abscessation, urinary tract infection, cellulitis, diarrhea, and bacteremia [2–6]. Furthermore, it is capable of causing nosocomial infections and high mortality rates are possible. *M. morganii* infections have been reported in a variety of domestic animals that are associated with humans, including broiler chickens, piglets, rabbits, and dairy calves [7–14]. It is noteworthy that *M. morganii* can live in the oral cavity of animals and thereby cause infections in humans via bites [3, 15–17]. Although the *M. morganii* can rarely cause the occurrence of zoonotic disease, it is noteworthy that *M. morganii* can spread in weak persons [18, 19].

Since the 1970s, *M. morganii* has been considered an important cause of nosocomial infections in adults, such as urinary tract and wound infections [2]. Some of these infections have had high mortality rates due to *M. morganii*'s virulence and increasing drug resistance [20]. *M. morganii* has also caused

disease in a variety of animals. *M. morganii* was isolated from chickens suspected with fowl typhoid or pullorum disease in Nigeria, and important pathologic lesions, including enlarged and congested spleens, enlarged livers congested and friable areas with necrosis, congested lungs, congested, mis-shaped and atrophic ovaries [21]. Large numbers of *M. morganii* were isolated from the pneumonic lesion of a piglet with serofibrinous pleuropneumonia [11]. *M. morganii* was isolated from the lungs, blood, liver, and blowhole mucosa of a dead dolphin with fibrino-hemorrhagic bronchopneumonia. This death was attributed to septicemia, based on the ecchymoses and petechiae of the spleen, pancreas, forestomach, lungs, visceral peritoneum, and small intestine [7]. In 2018, Li *et al.* reported a case of *M. morganii* infection in Holstein calves in Tai'an, Shandong province that originated from lactating cattle with latent infections. In this instance, calves were infected from the drinking milk and the presented clinical signs including depression, poor appetite, paralysis, and feces with white floccs [12]. Little is known about the prevalence of *M. morganii* in Chinese cattle, and this agent was not previously reported in beef cattle.

In the present study, prevalence of *M. morganii* in beef cattle was analyzed, three strains of *M. morganii* were isolated from cow nasal swabs in Guang'an, Yibin and Ziyang, Sichuan Province, China. Furthermore, the biochemical characteristics, drug sensitivity and pathogenicity of these *M. morganii* isolates were investigated.

Results

Morgenella morganii prevalence

Analysis data of the 191 samples showed that the prevalence of *Morgenella morganii* in three cities was 10.5% (20/191) the prevalence in Guang'an city was 18.2% (14/77) 7.8% (5/64) in Yibin, 2% (1/50) in Ziyang. The partial amplified positive PCR products were analyzed by agarose gel electrophoresis (Figure 1). Single DNA positive bands were clearly shown on the gel.

Morgenella morganii culture characteristics and 16S rRNA gene analysis

Three *M. morganii* strains (16GA7, 17GA61.2, and 17YB9) were isolated from positive nasal swab samples. Isolates grew as pale pink, translucent round small colonies on MacConkey medium (Figure 2A), and as white, translucent, weakly beta hemolyzed round colonies on blood agar medium (Figure 2B), belonged to gram-negative brevibacterium determined by gram staining (Figure 2C). The 16S rRNA gene of the strains were amplified and sequenced. Through the NCBI BLAST analysis, the similarity between the obtained sequences of isolates and reference *M. morganii* sequences in Genbank could reach 99%. The 16S rRNA gene sequences of *M. morganii* strains (16GA7, 17GA61.2, and 17YB9) have been deposited in the GenBank database under accession numbers MN807692, MN807693, and MN807694. These strains were phylogenetically clustered with *M. morganii* strains SQ-1 (GenBank accession number KJ794191.1) and IT-4-1 (GenBank accession number KU570304.1) (Figure 3). The three *M. morganii* strains had shared biochemical characteristics (Table 2); each strain could metabolize glucose, mannose, ornithine, and phenylalanine, could not metabolize sucrose, maltose, lactose, rhamnose, melibiose,

arabinose, mannitol, sorbitol, adonitol, inositol, lysine, arginine, esculin, and salicin, and was also H₂S test negative. Semi-solid agar puncture test showed that three *M. morganii* strains had no motility.

Antimicrobial susceptibility testing

Inhibition diameters and sensitivity for *M. morganii* strains 16GA7, 17GA61.2, and 17YB9 were shown in Table 3. Strain 16GA7 was sensitive to piperacillin, tetracycline, enrofloxacin, and dose-dependently sensitive to carbenicillin, meropenem, amikacin, and minocycline, and resistant to ampicillin, cephalixin, cefuroxime, imipenem, streptomycin, kanamycin, midecamycin, florfenicol and polymyxin B. Strain 17GA61.2 was sensitive to carbenicillin, piperacillin, amikacin, enrofloxacin, florfenicol, and dose-dependently sensitive to cefuroxime, meropenem, streptomycin, and kanamycin, and resistant to ampicillin, cephalixin, imipenem, tetracycline, minocycline, midecamycin and polymyxin B. Strain 17YB9 was sensitive to carbenicillin, kanamycin, tetracycline, minocycline, enrofloxacin, florfenicol, dose-dependently sensitive to piperacillin, streptomycin, amikacin, and resistant to ampicillin, cephalixin, cefuroxime, imipenem, meropenem, midecamycin and polymyxin B (Table 3).

Mouse virulence

Mice inoculated with *M. morganii* were depressed, huddled together, had abdominal breathing, and sticky ocular secretions, and moribund mice had open mouth breathing. No clinical abnormalities were evident in control mice. All mice inoculated with strain 16GA7 dead, 3 mice inoculated with strain 17GA61.2 dead, and no mice inoculated with strain 17YB9 dead. Pulmonary hemorrhage was evident in inoculated mice, with no other gross post-mortem pathology evident in other organs. Control mice had no gross pathological abnormalities (Figure 4). Each *M. morganii* strain was re-isolated from the tissue samples of mice inoculated with the corresponding strain.

Each *M. morganii* strain presented with similar histopathological findings, although differences were evident (Figure 5). Strain 16GA7 was associated with pulmonary hemorrhage, alveolar wall thickening, pulmonary inflammatory cell infiltration, spleen congestion, hepatic cord disorder, hepatocyte swelling and necrosis, and narrow renal cystic spaces. Strain 17GA61.2 was associated with pulmonary hemorrhage, necrosis, collapsed alveolar structures, pulmonary inflammatory cell infiltration, increased splenic multinuclear macrophages, indistinct splenic white pulp structure, hepatic cords derangement, hepatocyte swelling and necrosis, and narrowing of the renal cystic cavity (Figure 5). Strain 17YB9 was associated with pulmonary hemorrhage, alveolar wall thickening, pulmonary inflammatory cell infiltration, spleen congestion, vacuolar degeneration, hepatic cord derangement, hepatocyte swelling and necrosis, vacuolar degeneration; narrowing of the renal cystic space, and vacuolar degeneration. There were no abnormalities in the cardiomyocytes of the mice infected any of the *M. morganii* strains. There were no histopathological abnormalities in control mice.

Discussion

Morganella morganii can be an opportunistic pathogen when the host's immune system is compromised and the agent spreads systemically [22]. *Morganella morganii* is widely distributed in nature and infections have been reported in fish, amphibians, reptiles, birds, and mammals including humans [3, 23]. This agent has also been associated with food poisoning in humans [14, 24, 25]. Cattle farming has become a fast-growing industry in China. However, intensive beef cattle farming is facing challenges from infectious diseases. Our findings suggest that bovine *M. morganii* infections are a potential infectious disease concern for the cattle industry.

In our current study, the overall prevalence of *Morganella morganii* in Guang'an, Yibin and Ziyang was 10.5% (20/191). This result suggests that the risk of exposure to *M. morganii* in cattle is high in Sichuan. We isolated three *M. morganii* strains 16GA7, 17GA61.2 and 17YB9 from *M. morganii* positive nasal swabs collected from beef cattle farms in Sichuan. According to the medical history investigation, these cattle had a history of long-distance transportation shortly prior to disease onset. Transportation-related stressors and may have compromised immunity in these animals, and thereby favoring development of opportunistic *M. morganii* infections. A dolphin died from septicemia caused by *M. morganii* infection, and transport stress and introduction to a new facility were also considered to be the most likely risk factors [7].

All three *M. morganii* isolates were sensitive to quinolone and some penicillins-type antibiotics, and resistant to some cephalosporins, carbapenems, macrolide antibiotics and polymyxin B. The spectrum of antibiotics for which *M. morganii* has apparent resistance raises concerns for this agent becoming a multidrug-resistant or extensively drug-resistant zoonosis. A pediatric patient with sepsis was reported to have a *M. morganii* isolate that had NDM-1 and cephalosporinases, although this patient was managed successfully with a combination of aztreonam and ceftazidime-avibactam [26]. A Chinese cobra (*Naja naja atra*) isolate of *M. morganii* was reportedly susceptible to piperacillin [23]. *Morganella morganii* isolated from cattle experiencing high mortality was sensitive to streptomycin, imipenem, aztreonam, and cefoperazone [12]. Taiwanese patients had *M. morganii* isolates with resistance to first-generation cephalosporins and ampicillin-clavulanate [2]. Based on our results, we recommended using enrofloxacin or amikacin for treatment. Differences between our results and previous *M. morganii* studies drug resistance studies [2, 3, 12, 26, 27] may be caused by differences between individual strains and environment, including the application history of antibiotic use.

The virulence of *M. morganii* strains 16GA7, 17GA61.2 and 17YB9 differed, as did the pathology associated with each strain. Among these three *M. morganii* strains, 16GA7 had the strongest virulence, followed by 17GA61.2, and with 17YB9 having the least virulence. Pathology documented in infected mice included pulmonary hemorrhage, thickened alveolar walls, and pulmonary inflammatory cell infiltration, as well as varying degrees of splenic, hepatic, and renal lesions. These results differ from previous results for sdta1-sdta5 *M. morganii* isolates that were associated with hepatic and renal necrosis, hepatic nodules, and renal pelvis hemorrhage with the absence of pulmonary hemorrhage [12]. Comparative genomic analyses demonstrate that *M. morganii* pathogenesis varies with evolution of the agent's virulence [28]. Hosts are also likely more susceptible to *M. morganii* infection when they are

immunocompromised. In short, differences among *M. morganii* isolates need to be further characterized for an improved understanding of pathogenicity, appropriate diagnostic screening, vaccine development, and human public health control measures.

To the best of our knowledge, this study is the first to report the prevalence of *M. morganii* in cattle, and only one case report about dairy calves previously addressed *M. morganii* risks [12]. Nevertheless, *M. morganii* infections may have existed in cattle for a long term without being diagnosed or misdiagnosed. Therefore, *M. morganii* infections are of concern for the potential to become an emerging disease threat to the Chinese commercial cattle industry. Thus, this study is important for understanding the prevalence and pathogenic characteristics of *M. morganii*, and potential therapeutic antibiotics that may be effective for treating infections.

Conclusion

In our study, prevalence of *M. morganii* was surveyed and three *M. morganii* strains were isolated from beef cattle nasal swabs. In addition, our drug sensitivity assay results demonstrated that *M. morganii* was resistant to multiple antibiotics including cephalosporins, carbapenems, macrolide antibiotics and polymyxin B. Therefore, the development of appropriate management programs for *M. morganii* is vitally important and urgent for the Chinese commercial cattle industry. It is worth pondering that the risk of *M. morganii* infection in humans through contact with cattle or beef foods cannot be ignored.

Methods

Clinical sample collection

From April 2016 to October 2019, nasal swabs samples were collected from Sichuan Province beef cattle farms located in Guang'an ($n = 77$), Yibin ($n = 64$) and Ziyang ($n = 50$) (Table 1). Samples were collected from cattle with recently developed clinical signs following transport from other provinces. Clinical signs included nasal discharge, cough, and dyspnea.

Prevalence of *M. morganii* determined by PCR

DNA was extracted from nasal swab sample using a One-tube General Sample DNAup for PCR kit (Sangon Biotech (Shanghai) Co., Ltd.). The specific operation was carried out according to the instructions provided by the manufacturer, and the extracted DNA samples were stored at -20°C until analysis by PCR. The specific PCR primers Mm208F (5'-CTC GCA CCA TCA GAT GAA CCC ATA T-3') and Mm1017R (5'-CAA AGC ATC TCT GCT AAG TTC TCT GGA TG-3') were previously developed by Kim and others for detection of *M. morganii* based on the 16S rRNA sequence [29]. Primers were synthesized by Sangon Biotech (Shanghai). 2×Master Mix (Chengdu Qingke Zixi biotechnology Co., Ltd.) was used for amplification. *M. morganii* preserved in our lab was used as positive control in PCR. Pooled PCR products were confirmed by comparing DNA size with a DNA mass ladder (Chengdu Qingke Zixi biotechnology Co., Ltd.) on 1% agarose (Biowest, Spain) gel stained with ethidium bromide (Chengdu Haoboyou

biotechnology Co., Ltd.). Gels were placed on Gel Documentation system (Bio-Rad Laboratories) for band visualization and photography.

Isolation and identification of *M. morganii*

Each *M. morganii* positive nasal swab sample was placed in Tryptic Soy Broth (TSB) medium (Qingdao Hai Bo Biotechnology Co., Ltd.) for pre-enrichment. After incubation at 37°C for 24 h, one loop of each pre-enriched culture was streak-inoculated on to blood agar and MacConkey plates (Qingdao Hai Bo Biotechnology Co., Ltd.) and incubated at 37°C for an additional 24 h. Suspected single colonies were selected for microscopic examination with gram staining. Next, the suspected *M. morganii* purification cultures were identified using bacterial trace biochemical identification tubes (Hangzhou microbial reagent Co., LTD) and PCR amplification. Primer synthesis and PCR products sequencing were conducted by Sangon Biotech (Shanghai) using a 16S rRNA gene primer (Forward primer 5'-AGAGTTTGATCCTGGCTCAG-3', Reverse primer 5'-TACGGCTACCTTGTACGACTT-3'). Phylogenetic analysis was performed based on the 16S rRNA gene sequences available in the GenBank database. Phylogenetic trees were reconstructed using MEGA7.0 software [30], with sequences aligned to *M. morganii* reference strains retrieved from GenBank. Bootstrap values were tested with 1000 replicates using a neighbor-joining algorithm, and evolutionary distances were determined using the Kimura two-parameter model.

Antimicrobial susceptibility testing

The Kirby-Bauer disk diffusion method was used to determine *M. morganii* isolate antimicrobial susceptibility [31]. Three *M. morganii* isolates were tested to 16 antibiotics (ampicillin, carbenicillin, piperacillin, cefalexin, cefuroxime, imipenem, meropenem, streptomycin, kanamycin, amikacin, tetracycline, minocycline, midecamycin, enrofloxacin, florfenicol, and polymyxin B) using the Kirby-Bauer disk diffusion method. Results were interpreted following Clinical and Laboratory Standards Institute document M100-S28 guidelines [31].

Mouse virulence

4-week-old SPF-Kunming mice (n = 24, male, weight range = 18-22 g) were purchased from Chengdu Da Shuo Laboratory Animal Company (China). Mice were randomly assigned to four treatment groups with six mice in each group. Three treatment groups were inoculated with a unique *M. morganii* strain, and one group was set as control. Inoculations were performed using intraperitoneal injections of a suspension made from *M. morganii* cultures containing 4×10^8 CFU/mL (0.2 mL/per mouse) in a sterile common broth medium. Control group mice were intraperitoneally injected with equivalent volumes of sterile common broth medium. All mice were monitored for mortality and clinical signs such as ruffled fur, lethargy and dyspnea for 7 d post-inoculation. Mouse hearts, livers, spleens, lungs and kidneys were collected aseptically post-mortem and fixed in 4% paraformaldehyde (Solarbio Life Science) for routine processing and embedded in paraffin. Paraffin-embedded tissue samples were sectioned (3 μ m) and

stained with hematoxylin and eosin (H&E) (Beyotime, Beijing). Organ histopathology of all mice was documented by two pathologists who were blinded to treatments.

Abbreviations

morganii: *Morganella morganii*; S: Sensitive; S-DD: dose-dependent sensitivity; R: drug resistance.

Declarations

Ethics approval and consent to participate

The samples were collected from beef cattle in Sichuan Province, China. The cattle farm leader gave verbal consent for the sample collections. Because there were no vulnerable populations involved. This study was submitted to and approved by Animal Welfare Committee of Sichuan Agricultural University (Approval Number SYXK2019-187). The samples were collected and handled in accordance with the good animal practices required by the Animal Ethics Procedures and Guidelines of the People's Republic of China. Mice used in this study were handled in strict accordance with the guidelines of the Animal Welfare Committee of Sichuan Agricultural University. Mice were maintained in individual cages in a specific pathogen-free environment with an automatically-controlled 12-h light/dark cycle and free access to food and water for seven days. Then, they were randomly divided into different group. Mice were euthanized using sodium phenobarbital according to American Veterinary Medical Association guidelines (2013, AVMA) [32], and every effort was made to minimize animal suffering.

Consent for publication

Not applicable.

Availability of data and materials

The 16S rDNA gene sequences of 3 *M. morganii* isolates (16GA7, 17GA61.2, 17YB9) have been deposited in the GenBank database under accession numbers MN807692, MN807693, and MN807694, were free downloaded for scientific research. The other data information during the current study are available from the corresponding author (Zhikai Zuo, zzcjl@126.com) on reasonable request.

Competing interests

All authors declared no competing conflict of interest.

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Authors' contributions

BT and DJC analyzed the data and drafted the manuscript. YLZ, XL, YW, LPG conducted part of the experiment. JZL and MSW analyzed part of the data. ZJZ, JF, ZCZ participated in the conception and design the experiments and critically revised the manuscripts. All authors read and approved the final manuscript.

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Authors' information

¹Institute of Preventive Veterinary Medicine, Sichuan Agricultural University, Chengdu 611130, China

²Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Sichuan Agricultural University, Chengdu 611130, China

³College of Veterinary Medicine, Shandong Agricultural University, Tai'an 271018, China

⁴Ministry of agriculture "Veterinary medicine and veterinary technology" Sichuan scientific experimental observation station, Sichuan Agricultural University, Chengdu 611130, China

References

1. Morgan HDR. Report XCV. Upon the bacteriology of the summer diarrhoea of infants. *Br Med J*. 1906;1(2364):908–12.
2. Lin TY, Chan MC, Yang YS, Lee Y, Yeh KM, Lin JC, Chang FY. Clinical manifestations and prognostic factors of *Morganella morganii* bacteremia. *European Journal of Clinical Microbiology Infectious Diseases Official Publication of the European Society of Clinical Microbiology*. 2015;34(2):231–6.
3. Liu H, Zhu J, Hu Q, Rao X. *Morganella morganii*, a non-negligent opportunistic pathogen. *International Journal of Infectious Diseases*. 2016;50:10–7.
4. Nakajima M, Shirokawa M, Miyakuni Y, Nakano T, Goto H. Giant iliopsoas abscess caused by *Morganella Morganii*. *American Journal of Case Reports*. 2017;18:395.
5. Katz LM, Lewis RJ, Borenstein DG. Successful joint arthroplasty following proteus morganii (*morganella morganii*) septic arthritis: a four-year study. *Arthr Rhuem*. 2014;30(5):583–5.
6. Salen PN, Eppes S. *Morganella morganii*: a newly reported, rare cause of neonatal sepsis. *Academic Emergency Medicine Official Journal of the Society for Academic Emergency Medicine*.

- 2014;4(7):711–4.
7. Elfadl AK, Lee SW, Kim JH, Lee KL, Arif Ullah HM, Chung MJ, Ghim SG, Lee EJ, Kim YD, Kim SM. Fatal fibrino-hemorrhagic bronchopneumonia associated with *Morganella morganii* in a bottlenose dolphin: a case report. *Diseases of Aquatic Organisms*. 2017;127(1):41–7.
 8. Yu EQ, Zhang D, Li DM, Yang Z, Zai XS, Gong JS, Qin AJ, Jin WJ. **Isolation, identification and genetic evolution analysis of *Morganella morganii* from eggs.** *China Animal Husbandry & Veterinary Medicine* 2013, 8.
 9. Zhao Y, Li WJ, Du XB, Chen M, Kang K, Chen YL. Phylogenetic analysis and identification of biological characterization of *Morganella Morganii* in kangaroo. *China Animal Husbandry Veterinary Medicine*. 2010;37(3):48–51.
 10. Wani SA, Bhat MA. Antibiogram and bacterial flora of pyogenic infections of domestic animals in kashmir valley. *Indian Journal of Animal Sciences*. 2003;73(4):384–6.
 11. Ono M, Namimatsu T, Ohsumi T, Mori M, Okada M, Tamura K. Immunohistopathologic demonstration of pleuropneumonia associated with *Morganella morganii* in a piglet. *Vet Pathol*. 2001;38(3):336–9.
 12. Li G, Niu X, Yuan S, Liang L, Liu Y, Hu L, Liu J, Cheng Z: **Emergence of *Morganella morganii* subsp. *morganii* in dairy calves, China.** 2018, 7(1).
 13. Lei K, Zhu NY, Bei YJ, Ding XY, Chen JS. Isolation and identification of pathogenic *Morganella morganii* isolates from Chinese soft-shelled turtle *pelodiscus sinensis*. *Oceanologia Et Limnologia Sinica*. 2013;44(3):722–7.
 14. McCarthy S III. WB: Efficacy of electrolyzed oxidizing water against *Listeria monocytogenes* and *Morganella morganii* on conveyor belt and raw fish surfaces. *Food Control*. 2012;24(1–2):214–9.
 15. Chen CM, Wu KG, Chen CJ, Wang CM. Bacterial infection in association with snakebite: A 10-year experience in a northern Taiwan medical center. *J Microbiol Immunol Infect*. 2011;44(6):456–60.
 16. Chunyi L, Hsiufen L, Huang FL, Chen PY. Haemorrhagic bullae associated with a chicken scratch. *Ann Trop Paediatr*. 2009;29(4):309–11.
 17. Fischer J, Hille K, Mellmann A, Schaumburg F, Kreienbrock L, Köck R. Low-level antimicrobial resistance of *Enterobacteriaceae* isolated from the nares of pig-exposed persons. *Epidemiology Infection*. 2016;144(4):686–90.
 18. Di Ianni F, Dodi PL, Cabassi CS, Pelizzone I, Sala A, Cavirani S, Parmigiani E, Quintavalla F, Taddei S. Conjunctival flora of clinically normal and diseased turtles and tortoises. *BMC Vet Res*. 2015;11(1):91.
 19. Yacoub A, Janz T, Mojica L, Jones L, Greene JN. A fishmonger's tale: a case report and review of literature. *Infectious Diseases in Clinical Practice*. 2015;23(5):248–53.
 20. Lee IK, Liu JW. Clinical characteristics and risk factors for mortality in *Morganella morganii* bacteremia. *J Microbiol Immunol Infect*. 2006;39(4):328–34.

21. Mamman PH, Kazeem HM, Raji MA, Nok AJ, Kwaga JKP. Preliminary report on isolation of *Morganella Morganii* from fatal infections of chickens in Kaduna State. **Nigeria**. 2014;1(6):77–80.
22. Earley B, Sporer KB, Gupta S. Invited review: Relationship between cattle transport, immunity and respiratory disease. *Animal*. 2016;11(3):486–92.
23. Wang HF, Du LY, Luo J, He HX: **Isolation, identification and characterization of *Morganella morganii* from *Naja naja atra* in Beijing, China**. *Cellular and molecular biology (Noisy-le-Grand, France)* 2017, **63(7):52**.
24. Papadopoulou C, Economou E, Zakas G, Salamoura C, Dontorou C, Apostolou J. Microbiological and pathogenic contaminants of seafood in Greece. *J Food Qual*. 2007;30(1):28–42.
25. Fernández-No IC, Böhme K, Gallardo JM, Barros-Velázquez J, Calo-Mata P. Differential characterization of biogenic amine-producing bacteria involved in food poisoning using MALDI-TOF mass fingerprinting. *Electrophoresis*. 2010;31(6):1116–27.
26. Hobson CABS, Fahd M, Baruchel A, Cointe A, Poey N, Jacquier H, Doit C, Monjault A, Tenailon O: **Successful treatment of bacteremia due to NDM-1-producing *Morganella morganii* with aztreonam and ceftazidime-avibactam combination in a pediatric patient with hematologic malignancy**. *Antimicrobial agents and chemotherapy* 2019, **AAC-02463**.
27. Schultz E, Barraud O, Madec JY, Haenni M, Doublet B. Multidrug resistance *Salmonella* genomic island 1 in a *Morganella morganii* subsp. *morganii* human clinical isolate from France. *Msphere*. 2017;2(2):e00118-00117.
28. Chen YT, Peng HL, Shia WC, Hsu FR, Ken CF, Tsao YM, Chen CH, Liu CE, Hsieh MF, Chen HC. Whole-genome sequencing and identification of *Morganella morganii* KT pathogenicity-related genes. *Bmc Genomics*. 2012;13(7):1–14.
29. Leary SL, Underwood W, Anthony R, Cartner S, Corey D, Grandin T, Greenacre C, Gwaltney-Bran S, McCrackin M, Meyer R: **AVMA guidelines for the euthanasia of animals: 2013 edition**. In: *2013: American Veterinary Medical Association Schaumburg, IL; 2013*.
30. Kim SH, An H, Wei CI, Visessanguan W, Pitta TP. Molecular Detection of a Histamine Former, *Morganella morganii*, in Albacore, Mackerel, Sardine, and a Processing Plant. *J Food Sci*. 2006;68(2):453–7.
31. Sudhir K, Glen S, Koichiro T: **MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets**. *Molecular Biology & Evolution* 2016, **33(7):1870–1874**.
32. **M100 Performance Standards for Antimicrobial**. In.: Clinical and Laboratory Standas Institute 950 West Valley Road, Suite 2500 ….

Tables

Table 1 The Sample types, clinical syndrome, farm location and number of samples

Sample types	Clinical syndrom	Farm location	Number of samples
nasal swabs	respiratory symptoms	Guang'an	77
nasal swabs	respiratory symptoms	Yibin	64
nasal swabs	respiratory symptoms	Ziyang	50

Note: respiratory symptoms including nasal discharge, cough, and dyspnea.

Table 2 Biochemical test results for 16GA7, 17GA61.2, 17YB9 strains of *M. morgani*

Substrate	Results	Substrate	Results
Glucose	+	Mannitol	-
Sucrose	-	Sorbitol	-
Maltose	-	Adonitol	-
Lactose	-	Inositol	-
Mannose	+	Phenylalanine	+
Rhamnose	-	Ornithine	+
Melibiose	-	Lysine	-
Arabinose	-	Arginine	-
Semi-Solid Agar	-	Esculin	-
H ₂ S	-	Salicin	-

Table 3 Drug sensitivity test results for 16GA7, 17GA61.2, 17YB9 strains of *M. morgani*

Drugs	Inhibition diameters/mm			Sensitivity		
	16GA7	17GA61.2	17YB9	16GA7	17GA61.2	17YB9
Ampicilin	13.0	8.0	8.0	R	R	R
Carbenicilin	19.0	26.3	32.0	S-DD	S	S
Piperacilin	25.0	22.3	18.5	S	S	S-DD
Cephalexin	8.0	8.0	8.0	R	R	R
Cefuroxime	9.0	17.0	10.5	R	S-DD	R
Imipenem	14.0	13.5	10.0	R	R	R
Meropenem	15.0	16.0	14.0	S-DD	S-DD	R
Streptomycin	12.0	16.0	17.0	R	S-DD	S-DD
Kanamycin	11.0	19.8	22.0	R	S-DD	S
Amikacin	15.0	20.5	19.0	S-DD	S	S-DD
Tetracycline	23.0	11.0	21.8	S	R	S
Minocycline	18.0	9.5	20.7	S-DD	R	S
Midecamycin	8.0	8.0	8.0	R	R	R
Enrofloxacin	20.0	22.8	23.0	S	S	S
Florfenicol	8.0	25.3	24.3	R	S	S
PolymyxinB	8.0	8.0	8.0	R	R	R

Note: Sensitive (S); dose-dependent sensitivity (S-DD); drug resistance (R).

Figures

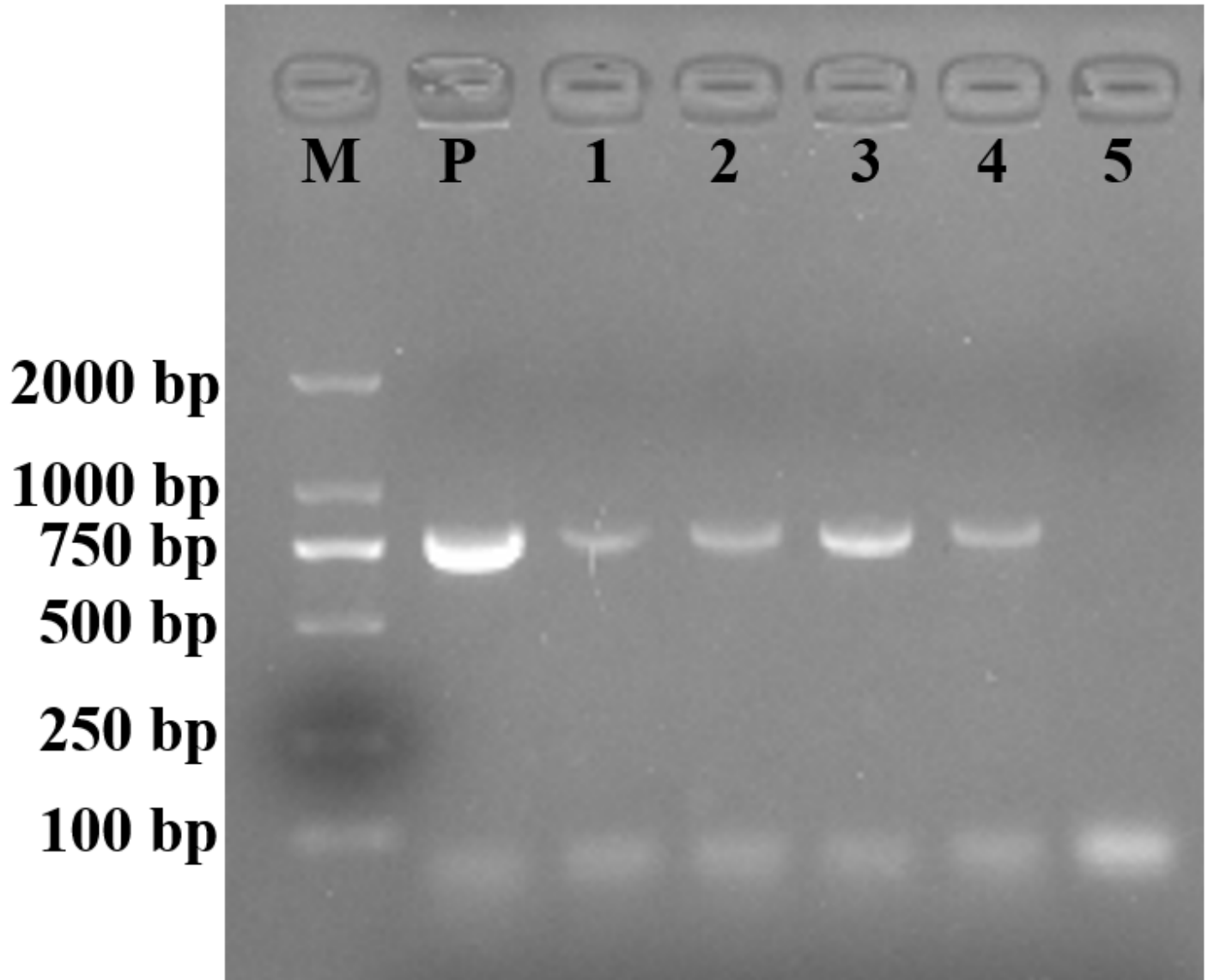


Figure 1

The partial amplified positive PCR products analyzed by agarose gel electrophoresis. M: DNA Marker, P: positive control, 1-4: samples PCR products, 5: negative control.

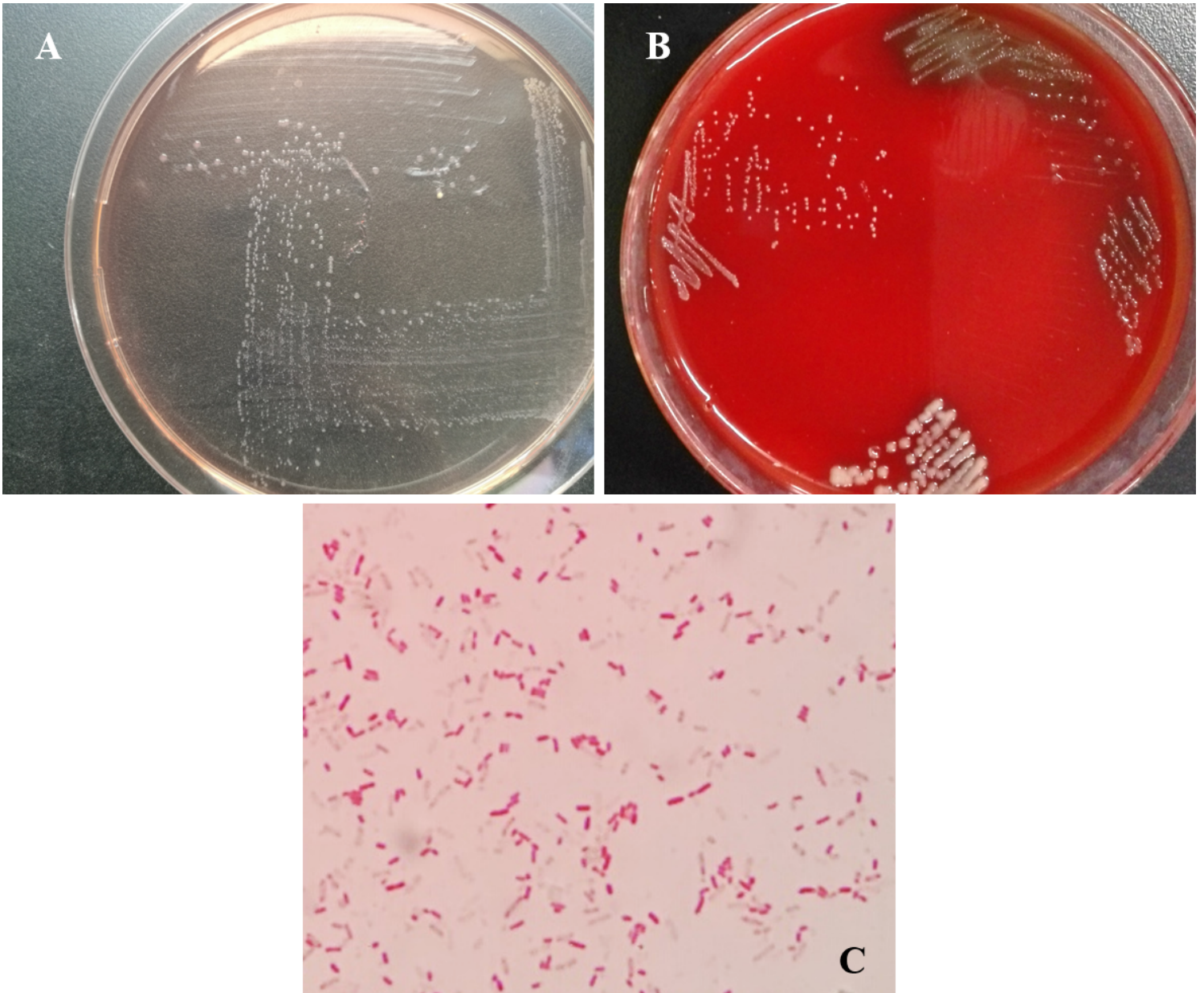


Figure 2

Morphology of *Morganella morganii* isolates. (A) Colony morphology of *M. morganii* strains in MacConkey agar. (B) Colony morphology of *M. morganii* strains in blood agar. (C) Microscopic morphology of *M. morganii* strains (1000 x).

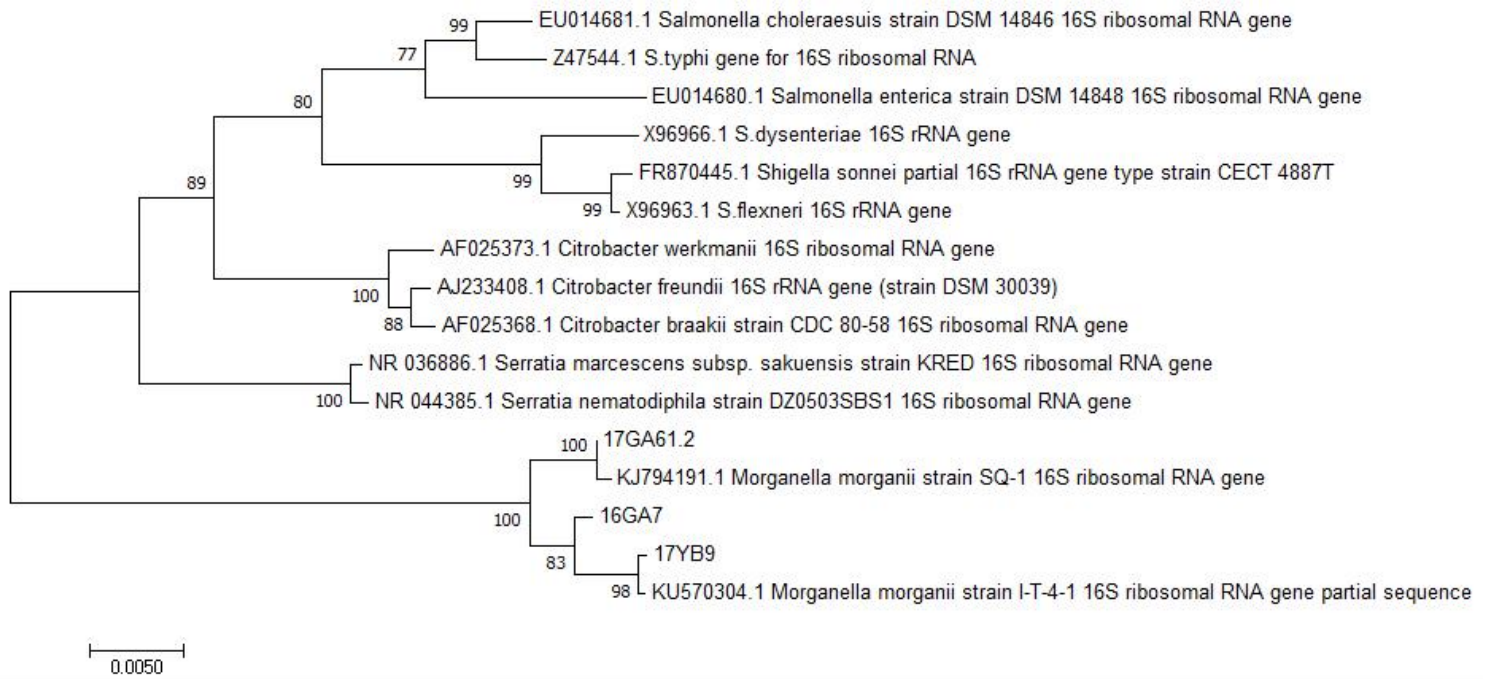


Figure 3

Phylogenetic tree analysis based on 16SrRNA gene sequences.

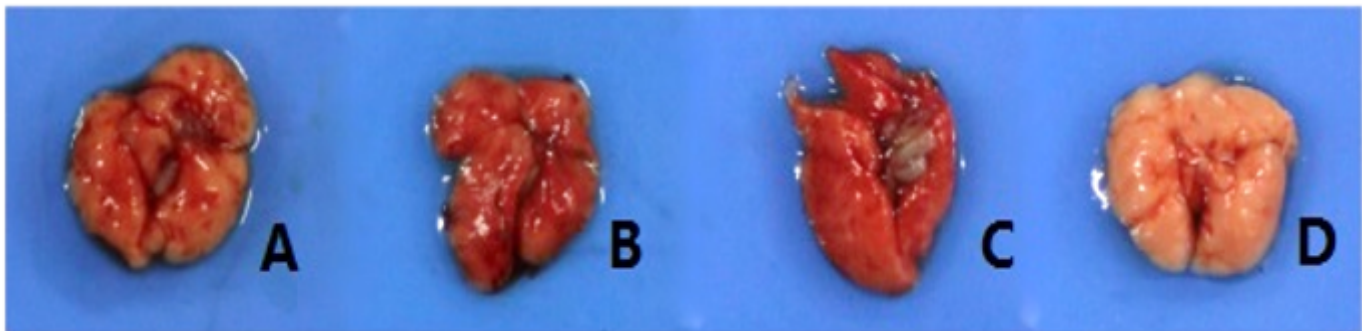


Figure 4

Representative gross post-mortem pulmonary tissues appearance for each treatment group. (A) 16GA7, (B) 17GA61.2, (C) 17YB9, (D) control group.

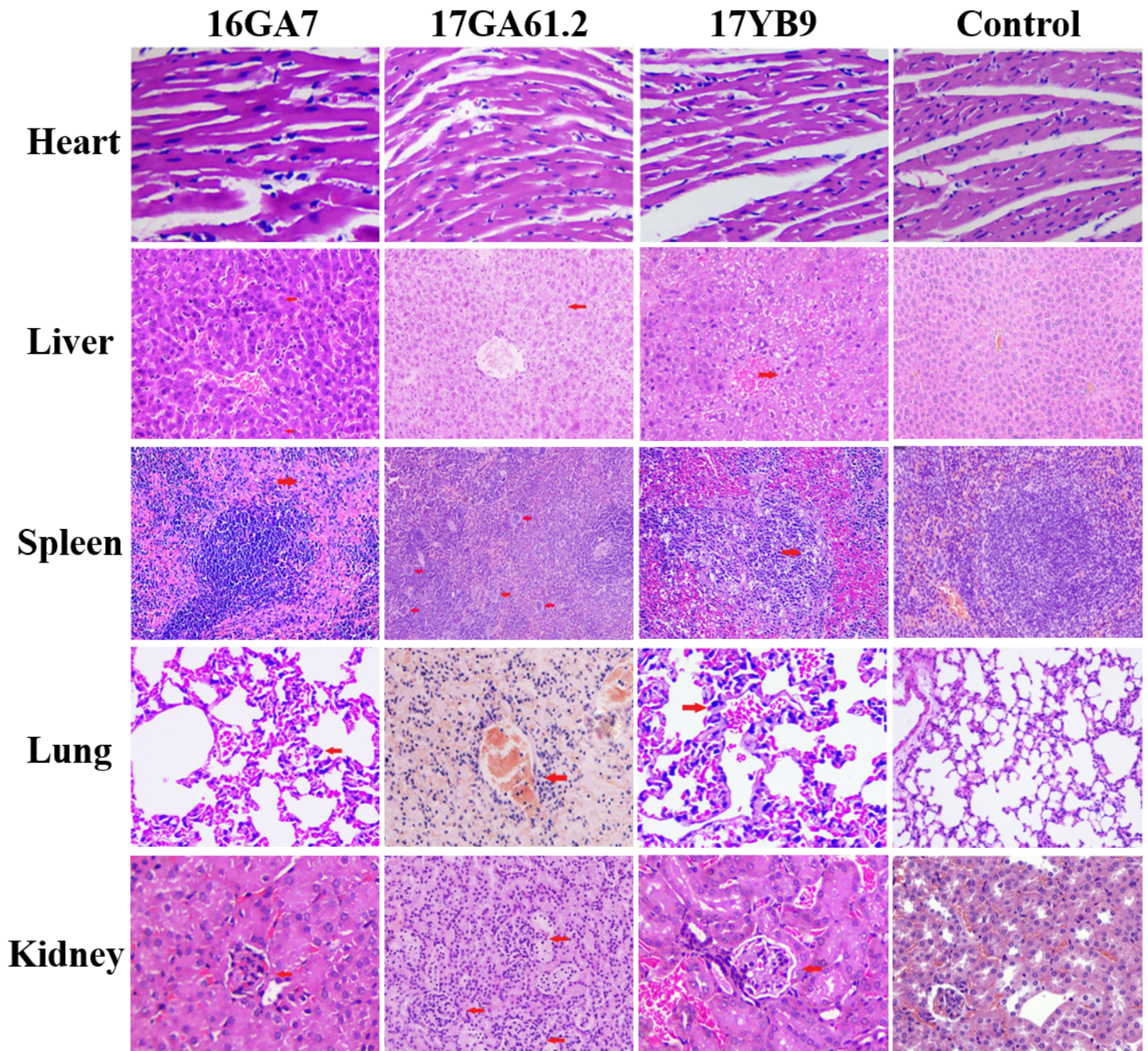


Figure 5

Representative photomicrographs of H&E-stained heart, liver, spleen, lung, and kidney sections (200x). Arrows for 16GA7, 17GA61.2, and 17YB9 photomicrographs identify areas of hepatocyte necrosis. In addition to hepatocyte necrosis, livers from 17YB9 group have vacuolar degeneration. The arrow for the 16GA7 group's spleen indicates splenic congestion, whereas the arrows for the 17GA61.2 group's spleen indicates multinuclear macrophage proliferation, and the arrow for the 17YB9 group's spleen indicates splenic congestion and vacuolar degeneration. The arrows for the 16GA7 and 17YB9 groups in lung tissue indicate areas of pulmonary hemorrhage, alveolar wall thickening and pulmonary inflammatory cell infiltration, and the arrow for the 17GA61.2 group indicates pulmonary hemorrhage. The arrows for the 16GA7, 17GA61.2, and 17YB9 group's kidney photomicrographs indicate narrowing of the renal

capsular space. In addition to narrowing of renal capsular space, the kidney for the 17YB9 group has vacuolar degeneration.

Supplementary Files

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- [NC3RsARRIVEGuidelines2013.pdf](#)