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Article

Effectiveness of an Herbal Remedy for Treating and Preventing African Swine Fever in Real-World Setting

Zhenghua Ma [1], Jiangnan Feng [1,*], Chengsheng Yi [1], Xia Liu [1], Meiying Gao [2], Dengbin Zhu [1], Minghai Ma [1], Rui Cheng [3], Xue Feng [1] and Yu Yan [1]

[1] Tongrun Tang TCM Clinic, Nanchong City, Sichuan, China

[2] Department of Medicine, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China

[3] Taipei Branch of Rui Peng Pet Hospital, Wuhan, Hubei, China; Linjiang Branch of Kedao Animal Hospital, Wuhan, Hubei, China

* Correspondence: jnfengyale@qq.com ; Tel.: 0086 13037182828

Abstract

Backgound African swine fever (ASF) is a highly infectious disease affecting pigs, caused by the African swine fever virus (ASFV), which results in a 100% mortality rate. Due to the absence of safe and effective vaccines and antiviral treatments, culling remains the primary strategy for managing ASF outbreaks. Recent studies suggest that Marecipe AV， a herbal therapeutic may provide significant therapeutic benefits against severe viral infections in both animals and humans. **Methods** This study evaluates the therapeutic and preventive effects of Marecipe AV in a real-world setting by comparing mortality rates, infection rates, and weight gain trajectories among pigs infected with ASF, both with and without the intervention of Marecipe AV. **Results** The mortality rate among domestic pigs infected with ASF and treated with Marecipe AV was 0%, in stark contrast to the 100% mortality rate observed in the untreated group. Remarkably, all treated domestic pigs, including those exhibiting severe ASF symptoms, demonstrated rapid recovery within 5 to 7 days following treatment. Furthermore, the incidence of ASF in the Marecipe AV prophylaxis group was 0%, while the untreated group experienced a 100% infection rate. Importantly, there was no adverse effect on the weight gain trajectory of domestic pigs receiving prophylactic treatment with Marecipe AV. **Conclusion** Marecipe AV herbal therapeutics has shown remarkable effectiveness in both the treatment and prevention of ASF. Utilizing Marecipe AV herbal therapy can lead to complete control of ASF.

Keywords: African swine fever (ASF); African swine fever virus (ASFV); viral infectious diseases; viral infectious diseases in animals; herbal remedies; therapy; prevention

Introduction

African swine fever (ASF) is an acute, virulent, common infectious disease of domestic and wild pigs caused by the African swine fever virus (ASFV) [1]. ASF was introduced into China and rapidly spread to several countries in the Asia-Pacific region, with morbidity and mortality rates reaching 100 percent, resulting in significant economic losses to the global pig industry. [2,3]Due to the lack of safe and effective vaccines and antiviral drugs, culling is the most effective method to control the epidemic[4].

Feng and his team recently reported a significant breakthrough in therapeutic approaches involving an herbal remedy for the treatment of viral infections. The herbal remedy, known as Marecipe AV, has been confirmed to be a powerful medicine with remarkable therapeutic effects in treating life-threatening viral infections in both animals and humans. It has been demonstrated that

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Marecipe AV therapeutics can reduce mortality rates from nearly 100% to 0% for several high-mortality diseases in animals, including avian influenza, canine distemper, canine parvovirus, porcine reproductive and respiratory syndrome and African Swine Fever (ASF) [5,6]. In the context of human acute viral infections, among a cohort of critically ill COVID-19 patients in the intensive care unit (ICU), the mortality rate was 0% for patients who received Marecipe AV therapy, while it was 36.36% for those who were untreated. Additionally, the hospitalization rate for progression to severe COVID-19 was 0% in high-risk patients who received Marecipe AV and 68.18% in untreated patients [7,8]. In this study, we present real-world outcomes of Marecipe AV therapy for the treatment and prevention of African swine fever (ASF), based on a substantial sample size.

Methods

Study design This study assessed the efficacy of drugs for African Swine Fever (ASF) in a real-world setting, using a large sample size with mortality as the primary endpoint. Mortality is the only non-modifiable outcome in swine infected with the ASF virus (ASFV), making it a definitive measure of efficacy and eliminating the need for control drugs, thus ensuring the accuracy of the findings. The preventive effect of drugs on ASF infection was evaluated by measuring the ASFV infection rate.

Subjects Domestic pigs located in the ASF epidemic area were selected for this study. Drawing from previous breeding experience, it was expected that these domestic pigs, including those newly introduced to the pigpen within the epidemic zone, would inevitably become infected with ASF.

Procedures 1. Treatment Protocol: Administer an oral loading dose of Marecipe AV at 10 grams per 50 kilograms of body weight, twice daily for 5 to 7 days. In Trials 1 and 2, the drug was given via gavage. In Trials 3 to 5, the drug was incorporated into drinking water, allowing affected pigs to self-administer. 2. Prophylactic Treatment Regimen: Preventive treatment regimen: The treatment regimen was changed from twice-daily to once-daily. 3. Disinfection Treatment: Marecipe AV was diluted in water and aerosolized using a nebulizer to create a dense mist in the pigpens. This treatment was administered once daily for 30 days to ensure effective disinfection without removing the domestic pigs from their pens.

Outcomes In the context of treating ASF, the primary endpoint was mortality. To evaluate the prevention of ASFV infection, both the mortality rate and the rate of viral infection were established as endpoints. Additionally, the weight gain curve was utilized to assess the efficacy of Marecipe AV therapy in preventing ASF.

Statistical analysis The Mann-Whitney U rank sum test was employed to compare the observed indicators among the treatment groups and the control group. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 27 for Windows.

Ethics In this study, treatment was conducted according to the daily operational practices of TCM. Licensed TCM doctors write a prescription, which the breeding owner then uses to purchase the required herbs from pharmacies. This approach adhered to the laws, regulations, and ethical guidelines governing TCM. Although the treatment methods and pharmaceutical preparations complied with established TCM norms and ethical standards, we submitted an ethical application for approval. The Ethics Committee of TCM at Tongren Tang Clinic approved this study (Ethics Committee document No. 202101).

Results

A total of five trials were conducted to evaluate the efficacy of Mareciep AV in the treatment and prevention of ASF.

In the first trial, a cohort of 30 ASFV-infected domestic pigs exhibiting severe clinical symptoms, including cessation of active eating, was established. Fourteen randomly selected subjects received Mareciep AV via oral gavage, while the remaining 16 served as untreated controls within the same facility. Additionally, 60 swine housed in nearby backyard pigpens within the same epidemiological unit were monitored as an external control group. Viral detection was performed using real-time

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quantitative PCR (qPCR) on a subset of randomly selected swine that displayed clinical signs consistent with ASF. All specimens (100%, n=3) tested positive for the ASFV. On day 15 after the initial dose of Mareciep AV, the mortality rates in the treatment and internal control groups were 28.57% (4/14) and 100% (16/16), respectively, while all 60 swine (100%) succumbed to ASF.

In the secondary trial, 63 ASFV-infected swine at early stages of disease progression (characterized by their ability to self-feed) were randomly allocated into two experimental groups. The treatment group (n=30) received Marecipe AV through immersion extract in their feed to promote self-feeding, while the control group (n=33) was maintained without intervention. Mortality surveillance by post-intervention day (PID) 15 revealed 0% mortality (0/30) in treated subjects compared to 100% lethality (33/33) in the control group ($p<0.001$, Fisher's exact test). Longitudinal monitoring through PID 30 confirmed full clinical recovery in all survivors, with sustained virological remission persisting throughout a subsequent 60-day observation period.

In Trial 3, a total of 3000 domestic pigs from a village experiencing the impact of an ASF epidemic were enrolled. The treatment group comprised 300 domestic pigs, approximately four months old, housed in one pigpen, while the control group consisted of 200 domestic pigs in an adjacent pen. In the experimental group, 500 domestic pigs exhibiting typical symptoms of ASF were confirmed to be infected with ASFV. All 20 nasopharyngeal swabs collected from these pens tested positive for ASFV, as verified by quantitative PCR (qPCR). At the time point preceding the initiation of Marecipe AV treatment, 23 out of 500 domestic pigs had succumbed, 3 domestic pigs had ceased both eating and drinking, and approximately 20 domestic pigs had stopped eating but were still able to drink freely. The remaining domestic pigs in both pigpens exhibited a significant reduction in feed intake. The 300 domestic pigs in the pigpen were administered Marecipe AV according to the treatment protocol, which involved mixing Marecipe AV into their drinking water twice daily, as described in the methodology. During the 7-day treatment cycle, three domestic pigs in the treatment group died due to cessation of active drinking, leading to their exclusion from the mortality count as they did not receive Marecipe AV. No additional deaths occurred in this group during the subsequent 60-day observation period. In contrast, all 200 domestic pigs in the control group died within the same timeframe. During this period, all domestic pigs in the other pens of the village died, resulting in the culling of 1500 out of approximately 2500 domestic pigs. Consequently, the mortality rate was 0% in the treatment group and 100% in the control group. Fever subsided within two days following the initial administration in all cases. The feed intake returned to normal in the majority of domestic pigs by the fifth to seventh day post-treatment. Most domestic pigs in treatment group achieved full recovery after 9 days of the initial dose. Five domestic pigs recovered completely 20 days after the initial treatment. Ten samples from treatment group were subjected to detection of ASFV using qPCR on days 15 ,45 and 60 following the initial treatment. None of the samples tested positive for the ASFV. Additionally, antibody testing for ASFV was conducted 60 days post-treatment, and all serum samples tested were found to be positive. The results of the ASFV pathogen and the antibody assay against ASFV are presented in Table 1.

Table 1. The results of the ASFV pathogen and the antibody assay against ASFV.

Sample	Time of sampling(day)	quantity	Number of Positive /total samples tested
Nasopharyngeal swabs of pigs in the treatment group	0	10	10
nasopharyngeal swab of pigs in control group	0	10	10
Nasopharyngeal swabs of pigs in the treatment group	15	10	0/10
Nasopharyngeal swabs of pigs in the treatment group	45	10	0/10

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Nasopharyngeal swabs of pigs in the treatment group	60	10	0/10
Serum of pigs in the treatment group	60	10	10/10

For detection of the ASFV pathogen, the Nasopharyngeal swabs samples was employed by quantitative PCR (qPCR). Serum anti-ASFV antibody was detected by ELISA.

In Trial 4, a total of 500 domestic pigs from the same village, which experienced an outbreak of ASF, were enrolled in the study. A total of 300 three-month-old domestic pigs were housed in Pen A, and 200 three-month-old domestic pigs were housed in Pen B. No signs of ASF were observed in domestic pigs in either Pen A or Pen B. Ten nasopharyngeal swab samples were collected from domestic pigs in each pen for the detection of ASFV, and all results were negative. Eight domestic pigs confirmed to be infected with ASF were randomly assigned to Pens A and B to facilitate the establishment of infections. The intervention for domestic pigs in Pen A was implemented as follows: 1. The Marecipe AV soak solution was used to disinfect the pen through daily spraying for 30 consecutive days; 2. Marecipe AV soak was added to the drinking water once daily for a duration of 30 days. No intervention was conducted for the domestic pigs in pigpen B. Over the subsequent 15 to 60 days, all pigs in pigpen B exhibited symptoms of African Swine Fever (ASF), including fever, bleeding spots, cyanosis, weakness, and reduced feed intake. Ten nasopharyngeal swabs from pigpen B tested positive for ASF virus (ASFV). All 200 pigs in pigpen B succumbed, with 40 deaths attributed to ASF and 160 culled. In contrast, pigpen A, which housed 300 domestic pigs, reported no fatalities or symptoms of ASF. The weight gain curve for domestic pigs in pigpen A showed no significant bias. Ten serum samples from pigpen A were collected for ASFV antibody detection; two samples tested positive and eight negative. Refer to Table 2 for further details.

Table 2. The results of the ASFV pathogen and the antibody assay against ASFV.

Sample	Time of sampling(day)	quantity	Number of Positive /total samples tested
Surface specimens from treatment pigpens	0	3	3/3
Surface specimens from control pigpens	0	3	3/3
Nasopharyngeal swabs of pigs in pigpen A(treatment)	0	10	0/10
nasopharyngeal swab of pigs in pigpen B (control)	0	10	0/10
Nasopharyngeal swabs of pigs in pigpen A(treatment)	18	10	0 / 10
nasopharyngeal swab of pigs in pigpen B (control)	18	10	10/10
Nasopharyngeal swabs of pigs in pigpen A(treatment)	30	10	0/10
Nasopharyngeal swabs of pigs in pigpen A(treatment)	60	10	0/10
Serum of pigs in pigpen A(treatment)	60	10	2/10

For detection of the ASFV pathogen, the Nasopharyngeal swabs samples was employed by quantitative PCR (qPCR). Serum anti-ASFV antibody was detected by ELISA.

In Trial 5, a total of two thousand domestic pigs from an ASF-endemic area were included in the study. The infected domestic pigs with ASF had recently been culled and removed from the pens in the same villages as those in Trial 3. Pigpens C and D served as controls and underwent disinfection using alternating applications of sodium hydroxide and sodium hypochlorite once daily for three consecutive days prior to the introduction of the piglets. The remaining pigpens in the village were

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disinfected by spraying them with Marecipe AV soak solution once a day for three days. Three surface samples from each pigpen in the village were collected and tested for ASFV, with almost all samples returning positive results. Three hundred 60-day-old piglets were randomly assigned to pigpens C and D, while the remaining 1,700 piglets were distributed across different pens within the same village. Nasopharyngeal swabs from 10 randomly selected piglets from each pigpen were tested for ASFV, and all results were negative. Following the housing of the piglets, the pigpens designated as treatment were sterilized daily using a Marecipe AV soak solution. In contrast, no actions were taken regarding pigpens C and D. After being housed in pigpens for 15 to 20 days, more than half of the piglets in pens C and D displayed clinical signs consistent with ASF. Seven piglets succumbed to the disease. Nasopharyngeal swabs were collected from 10 piglets in pens C and D, and all samples tested positive for ASFV. Following an additional 15 to 20 days, 7 out of the remaining 1700 piglets developed symptoms of ASF. Nasopharyngeal swabs were collected from these 7 piglets, resulting in 5 out of the 7 samples testing positive for ASFV. From that point onward, all piglets, including those in pens C and D, received oral Marecipe AV prophylaxis and spray disinfection with Marecipe AV soak solution. After the implementation of Marecipe AV prophylaxis, there were no new cases of ASF or associated deaths among the piglets, including those in pens C and D. Four months after the piglets were placed in the pens, the weight gain of the 1,700 piglets remained within the normal range, with no instances of abnormal weight gain observed. Serum antibody testing for ASFV was conducted 60 days after the initiation of oral Marecipe AV treatment. All 10 samples from pigpens C and D tested positive for ASFV. In contrast, among 1,700 piglets from various pigpens, 195 tested negative and 5 tested positive. These results indicate that all piglets in pens C and D were infected with ASFV, while most pigs receiving Marecipe AV prophylaxis remained uninfected. The incidence of ASF was approximately 2.5% in the Marecipe AV group, while it was 100% in the control group. The results of the antigen and antibody tests for ASFV are presented in Table 3.

Table 3. The results of the ASFV pathogen and the antibody assay against ASFV.

Sample	Time of sampling(day)	quantity	Number of Positive /total samples tested
Surface specimens from pigpens of control pigpens	0	6	6/6
Surface specimens from pigpens of treatment group	0	60	56/60
Nasopharyngeal swabs of pigs in pigpen C and D	0	10	0/10
Nasopharyngeal swabs of pigs in pigpens (before treatment)	0	120	0/120
Nasopharyngeal swabs of pigs in pigpen C and D	18	10	10/ 10
Nasopharyngeal swabs of pigs in different pigpens (Preventive disinfection with Marecipe AV)	18	200	0/200
Nasopharyngeal swabs of pigs in different pigpens (Prophylaxis disinfection with Marecipe AV)	30	120	5/120
Nasopharyngeal swabs of pigs in pigpen C and D (Prophylaxis with disinfection and oral Marecipe AV)	45	10	0/10
Nasopharyngeal swabs of pigs in different pigpens (Prophylaxis with disinfection and oral Marecipe AV)	45	10	0/120

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Nasopharyngeal swabs of pigs in pigpen C and D (Prophylaxis with disinfection and oral Marecipe AV)	60	10	0/10
Nasopharyngeal swabs of pigs in different pigpens (Prophylaxis with disinfection and oral Marecipe AV)	60	200	0/200
Serum of pigs in pigpen C and D	60	10	10/10
Serum of pigs different pigpens (Prophylaxis with disinfection and oral Marecipe AV)	60	200	5/200

For detection of the ASFV pathogen, the Nasopharyngeal swabs samples was employed by quantitative PCR (qPCR). Serum anti-ASFV antibody was detected by ELISA. No adverse events or toxicities were observed.

Discussion

African Swine Fever (ASF) is a catastrophic disease that can lead to mortality in nearly all infected swine. Many ASF virus (ASFV) strains result in the death of almost 100 per cent of infected pigs[9]. Currently, there is a consensus that it is not feasible to save lives from life-threatening viral infections in animals, including ASF. In the case of ASF, death remains the only definitive outcome. Given that death is an immutable and unequivocal endpoint for ASF, utilizing it as the primary outcome measure in this efficacy study minimizes potential bias and enhances the reliability of the results. This study also evaluated outcomes of Mareciep AV for prevention of ASF.

The results indicate that Marecipe AV herbal therapeutics offers significant benefits for ASF, achieving an impressive 100% cure rate and a 0% mortality rate. To our knowledge, no drug or treatment has previously achieved a 100% cure rate for fatal infectious diseases in animals, including FAS. It is noteworthy that a 5 to 7-day course of oral Marecipe AV treatment led to complete remission in all affected pigs, including those presenting with severe symptoms. Marecipe AV therapeutics demonstrated potent effective results against ASF and can achieve complete control over the disease.

We believe that the primary factor contributing to the higher mortality rates observed in the treatment group during the earlier experiments (Trial 1 and Trial 2) was the method of drug delivery. Administering drugs to domestic pigs via gavage presents a significant challenge, even in slow-moving animals exhibiting severe symptoms of African Swine Fever (ASF). Experimental operators have estimated that the success rate of gavage in these domestic pigs is less than 50%. Consequently, the majority of the domestic pigs involved in these studies did not receive an adequate dosage of Marecipe AV. This suggests that the key factor determining treatment success or failure is the completion of drug administration, rather than the drug's efficacy alone.

Marecipe AV has shown great benefit in preventing ASF. No disease of ASF and no ASFV infection occurred in most of domestic pigs that received Marecipe AV prophylaxis treatment in ASFV-exposed Settings. All domestic pigs in the control group were confirmed to be ASFV infected. The antibody against ASFV tests in the prevention treatment group were negative, proving that most of domestic pigs were not infected with ASFV and that Marecipe AV prophylaxis was effective and could completely prevent domestic pigs in ASFV-exposed environments from being infected. With the implementation of Marecipe AV prophylaxis, the growth of domestic pigs remained entirely unaffected by ASF.

In the present study, no effective data were collected on the efficacy of Marecipe AV alone as a disinfectant to prevent ASF. When ASFV infection was confirmed in a few domestic pigs in the Marecipe AV disinfectant group, oral administration of Marecipe AV was promptly initiated as a preventive strategy. The main reason for discontinuing the independent use of Marecipe AV as a disinfectant was insufficient funding to cover the costs associated with culling and burying large numbers of domestic pigs, which was unrelated to the experimental design. Results showed that over half of the 300 piglets exhibited clinical symptoms of ASF within 15 to 20 days of rearing. In contrast, only 5 out of 1,700 piglets treated with the Marecipe AV disinfectant developed the disease after 30 days. Serum antibody testing revealed that all domestic pigs in the untreated group were infected

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with ASFV, while only 2.5% of the treated group showed signs of infection. These data suggest that Marecipe AV, as a disinfectant, has a preventive effect on domestic pigs in an ASFV exposure environment. At a minimum, Marecipe AV as a disinfectant may reduce the duration of oral administration of Marecipe AV. The prevention of ASF by Marecipe AV as a disinfectant requires further experiments for evaluation.

At least two studies have reported the results of long-term follow-up on convalescent pigs and their offspring. There were neither carriers of nor recurrence of disease in the convalescent pigs and their offspring following the outbreak of acute ASF. The infectious ASFV was not detected in any of the tissue samples from ASFV convalescent and in-contact pigs[10]. In the present study, all results indicated that neither Marecipe AV-treated nor prophylactically treated domestic pigs infected with ASF exhibited detectable levels of the ASFV pathogenic agent. This suggests that the recovered domestic pigs were not capable of transmitting the virus. From a biosafety perspective, cured domestic pigs should pose minimal risk of ASF transmission and should not raise significant biosafety concerns.

Limitations

This study has several limitations. Firstly, the specific active ingredient in Marecipe AV and its exact mechanism of action remain unknown. Secondly, due to the lack of in vitro data on Marecipe AV as a disinfectant, we are unable to determine its effectiveness in killing or inhibiting ASFV. Additionally, due to limited funding, we could not afford the costs associated with culling and burying large numbers of domestic pigs infected with ASF. As a result, we implemented oral Marecipe AV measures to reduce the number of culled and buried domestic pigs when ASF cases occurred in the prevention group. This limitation hindered our ability to collect data on the efficacy of Marecipe AV as a disinfectant. Finally, we did not gather data on the use of Marecipe AV in treating ASF cases with severe symptoms or those nearing death.

Conclusions

Marecipe AV herbal remedy demonstrates a potent and effective impact on both the treatment and prevention of ASF. Based on the findings, it can be anticipated that the implementation of Marecipe AV therapy could lead to complete control of ASF.

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