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# Development and experimental evaluation of formalin killed vaccine for prevention of swine erysipelas outbreaks in North East India

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ABSTRACT

*Erysipelothrix rhusiopathaie*, a gram positive bacterium is responsible for a lethal disease popularly known as swine erysipelas in swine throughout the pig rearing countries including India. Being a zoonotic disease it is not only a concern from swine industry but also for human health. In case of outbreak in pigs, it causes huge mortality in unvaccinated population. Though most of the further loss can be controlled by penicillin group antibiotics but the disease can cause devastating loss to the farm in case of delay in diagnosis and treatment. Though there is vaccine available in global market but for Indian pig industry the vaccine is not available. We have been able to show the presence of the pathogen in the state of Meghalaya through serological, molecular, outbreak investigations in earlier studies and the disease is well present in other pig growing states of India including North East. The present study is focused on development of an effective formalin killed vaccine for regional preparedness against the dreaded pathogen. The prepared vaccine was able to show sero-conversion with as high as three times in the experimental pigs following a single booster in the pilot scale study. The same vaccine protocol can be used for preparation of vaccine in regional laboratories including North east states of India where pig husbandry is one of the main livelihood sources of farmers using the ATCC strains as used in the current experiment or their own indigenous isolates.

## 1. Introduction

Erysipelas in pigs is one of the major bacterial diseases encountered in the pig growing countries including India. The disease had been reported from all over the country where pig industry is existing (Char *et. al.,* 1993, Saini *et. al.,* 1994) with reports from North East India also (Kumaresan *et. al.,* 2009; Das *et. al.,* 2014, Das *et. al.,* 2020, Barman *et. al.,* 2016). The causative agent, *Erysipelothrix rhusiopathiae* is a gram positive rod which is responsible for causing skin disease in pigs and human, being a zoonotic pathogen. The organism is also responsible for arthritis and endocarditis is pigs and human. The bacterium is difficult to isolate and to maintain also in laboratory. It requires blood based agar and micro-aerophillic environmental to grow on laboratory media. The organism form very tiny colonies smaller than that of *Staphylococcus aureus* on the agar based media and may often get unnoticed during isolation process. The growth also took minimum of 48 hours during primary culture isolation and many a times the other rapid growing bacteria hinder its isolation process. The bacterium is not usually supplied by most of the companies and repository based institutes because of difficulty in isolation and maintenance of the bacterium. Besides the routine culture isolation and identification through bio-chemical/ molecular PCR based methods, ELISA based serological kits are also available for sero-survey works. Pig vaccine against *E.rhusiopathiae* is available in the world market and is also being practiced in major pig rearing countries but the vaccine is not available in Indian market. The disease cause heavy outbreaks in the pig farm and if not diagnosed and treated with penicillin group of antibiotics than it causes heavy mortality. Its differential diagnosis is needed from classical

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swine fever, porcine reproductive and respiratory syndrome, piglet anemia and other haemmorhagic diseases. All the mentioned diseases are endemic in India including in North Eastern states and now the NE and other part of Indian states had also seeing major outbreaks of African Swine Fever. Hence, usually the *E.rhusiopathaie* outbreak got confused with these endemic diseases and is under-reported. The disease is also reported to be seasonal or when there is a break in bio-security measures in the farms. The unavailability of vaccine in the country made us to start to work to produce a vaccine or its protocols. Hence, we attempted to make first a simplest killed vaccine and showed its sero-conversion in the animal experimentation as a measure of preparedness to tackle this disease in future in the country including North East. Here we will show the protocol of vaccine used so that the other researcher/laboratories can follow it as a measure of preparedness for preventing such outbreak or to carry out ring vaccination during outbreak time to control the spread of the outbreak

## 2. Methodology

**Procurement, revival and confirmation of culture-** The strain *Erysipelothrix rhusiopathiae* (Migula) Buchanan SKU ATCC 35456 was maintained in beads of Microbank by Pro-Lab Diagnostics, Canada at division of animal and fisheries sciences at deep freezer (-20 degree celsius). Two to three beads were taken with the help of sterile scalpel and were re-suspended in 1ml of Tryptose soy broth (TSB). It was further added to two 5ml tubes of TSB. The tubes were put in shaker incubator at 37ºC for 48 hours under 5% Carbon-dioxide (CO2) and the growth of bacteria was observed as turbidity. A loopful from both the tubes was taken for gram staining after the incubation. Upon confirmation for growth and purity through microscopic visualizations of the stained slides, another loopful were taken from both the broth and were streaked on Blood Agar plates with incubation at 37ºC for 48 hours under 5% CO2 to check colony purity. The work had been carried out in biosafety level 3 cabinets with biosafety level 2 facilities of laboratory of animal health, ICAR RC NEH Umiam.

To further confirm the presence of organism, the DNA was isolated from broth and colony by using Bacterial DNA isolation Kit (Qiagen,USA). Later on the isolated DNA, *Erysipelothrix* sp. PCR system using oligonucleotide primers complementary to the DNA sequence coding for 16S rRNA was used for testing as per Makino *et al.* 1994 (primer sequence- MO101-F: AGATGCCATAGAAACTGGTA; M0102-R: CTGTATCCGCCATAACTA) which amplify a 440bp product from *Erysipelothrix* DNA using Thermal Cylcer (eppendorf, Germany). The PCR reaction mixture was made in a volume of 25 µl comprising of 2.5 µl (without MgCl2) of 10X PCR buffer, 4.5 µl of 25mM $MgCL_2$, 2.0 µl of 200µM dNTPs, 1µl of 10 picomol of each primer, 1 unit of Taq DNA polymerase and 2µl of sample DNA. Cycling condition consisted of one cycle of initial denaturation at $95^0$C for 5 min, followed by 30 cycles of (denaturation at $95^0$C for 30 s, annealing at $54^0$C for 1 min and extension at $72^0$C for 30 s) with final extension at $72^0$C for 5 min. Finally, agarose gel electrophoresis was done for the PCR product and visualised under gel documentation system for bands of DNA of appropriate size.

**Bulk culture growth-** The 5 ml confirmed culture was then transferred to 100 ml TSB and was incubated for shaker incubator at 37ºC for 48 hours under 5% CO2. Though this quantity was sufficient for experimental purpose but if there is more requirement than the 100ml can be used as inoculum for further more broth say 01 litre. We will be keeping discussing our further experiment on 100 ml growth culture only. To this 100ml growth we have added 0.5% formaldehyde analytical grade and kept the solution for 12 hour at 37ºC to act. It should be noted that any compromise of quality of the formaldehyde will not be able to kill the bacteria. Thereafter, we did sterility test in two different media viz., tryptose Soy Agar and blood agar and the plates were kept in two different incubators at 37ºC for 48 hours under 5% CO2. Maintenance of two different media or incubator is essential to remove any human or instrumental errors. If there is a growth after incubation than we have to repeat the formalin step once more and again we have to do the sterility test. In case of *E.rhusiopathiae* we have repeated this killing step twice or thrice to ensure no organism is survived. This is one of the most critical steps and had to be under with keen observation. Thereafter, concentration was checked according to McFarland No i.e., Mc no 4 – 1.2 x $10^9$ or with in ELISA plate by adding around 100µl of product with reading at O.D 600nm. We further dilute the solution by increasing the addition of Normal Saline Solution to make it Mc no 3 – 9.0 x $10^8$. To this add 2% Aluminum hydroxide (autoclaved) as adjuvant and a final sterility test was carried out. Now the vaccine is ready for animal experimentation. The vaccine should be first checked in laboratory animals like mice or rabbit and then later can be applied to the host animal i.e., pigs. We have directly used the animal experimentation in small group of pigs (n=3) as we have animal ethical approval for pig only and had an experimental farm for such purpose. After the initial results of small group we expanded our farm experiment further to a bit large group for final recoding of sero-conversion.

**Animal experimentation-** Piglets of above 3 months age were taken and were maintained in the pig farm of ICAR RC NEH Umiam with ad-lib food, water and under best management practices. The animal experiments were duly approved by

institute animal ethics committee, approval number-IAEC/2020/7. In group 1 (3 animals) were given 2 ml vaccine plus one booster at 42 days of initial dose through I/M route and another group 2 (1 animal) was given through S/C route and two animals were kept as control in Group 3. The animals were kept in same managemental setup and were observed for their health status in regular interval. Their bloods were collected aseptically in serum vacationer (BD) at 0, 21, 63 and 90 day. The serum vacationer were kept undisturbed in the farm itself for 30 min and were transported to laboratory under refrigerated condition where sera was obtained and were stored at -80°C until tested.

**ELISA for sero-conversion-** Ingezim Mal Rojo indirect ELISA (Spain) was used for testing the sero-conversion following the manufacturer protocol and the reading was recorded by ELISA reader (Tecan life sciences). All the ELISA tests were repeated thrice to check the repeatability and for confidence in the results. The whole experiment from revival of strain, vaccine production, and animal experimentation to ELISA was conducted from the year 2019-2022 at Division of Animal and Fisheries Sciences, ICAR RC NEH, Umiam, Meghalaya.

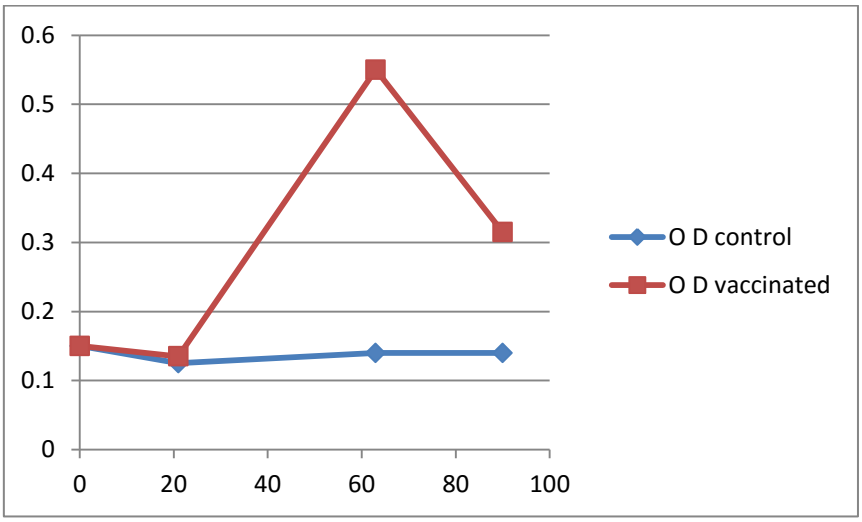
## 3. Results and Discussion

The *Erysipelothrix rhusiopathiae* ATCC 35456 strain was revived in broth and culture and was found pure during gram's staining. The culture showed required band during confirmation by PCR showing 440 band size using Makino et al., 1994. The vaccine prepared as per the protocol passed through the sterility test after the formaldehyde application in both the duplicate settings of media and different incubators. The vaccine also passed through the next sterility test after application of alum adjuvant in duplicate settings. The vaccine after application to the piglets does not showed any adverse reaction and when tested for sero-conversion did not showed sero-reactivity at day 21 initially but later showed sero-conversion at day 63 (21 days after booster at day 42) with more than 3.7 times than the non-vaccinated animals. The sero-conversion came for both intra-muscular and sub-cutaneous (S/C) route with more pronounced sero-conversion for S/C route. The animals were further observed for next 1 month (day 90 from initial dose) and more than two times the titer was maintained than control animals.

**Table 1-** Animal experimentation with 0 day vaccine, 42 day booster, sero-conversion at day 63, 90 days results for vaccinated and control group

| Animal number | Vaccine/control | Dose at Day 0 | Day 21 | Booster at Day 42 | Day 63 | Day 90 |
|---|---|---|---|---|---|---|
| **3234** | Vaccine | 2 ml [a]i/m | No [d]SeC | 2 ml i/m | [d]SeC ++ | SeC + |
| **3238** | Vaccine | 2 ml i/m | ---do--- | 2 ml i/m | SeC++ | SeC + |
| **3236** | Vaccine | 2 ml i/m | ---do--- | 2 ml i/m | SeC++ | SeC + |
| **3232** | Vaccine | 2 ml [b]s/c | ---do--- | 2 ml s/c | SeC ++++ | SeC ++ |
| **41190** | Control | [c]DW | ---do--- | DW | No SeC | No SeC |
| **41189** | control | --- | ---do--- | --- | No SeC | No SeC |

[a] i/m : intra-muscular, [b]s/c : sub-cutaneous, [c]DW : distill water, [d]SeC : sero-conversion




X-axis= days, Y-axis= Mean O.D of ELISA

**Figure 1.** Sero-conversion data for the piglets vaccinated with killed *Erysipelothrix rhusiopathiae* vaccine

*E.rhusiopathiae* is a fatal disease of pigs if untreated and not diagnosed properly. The disease in field condition is difficult to diagnose and is of zoonotic significance. The disease is worldwide present in the pig rearing countries including in India and North-East India which is the largest pig rearing and consuming belt in India. Though there is a vaccine available in global market but the vaccine is not available in India and neither any vaccine technology for this pathogen is available in the country. Hence, this vaccine protocol is attempted for future disease preparedness. The formalin killed alum adjuvant vaccine made using ATCC strain showed sufficient sero-conversion and is tested upto 90 days of vaccination. The vaccine protocol is simple and can be used by different institutes/biological production in the country. The vaccine is working both through intra-muscular and by sub-cutaneous mode with 2 ml dose with sub-cutaneous mode being showing better sero-conversion. The limitation of the study is that we have taken a smaller group for live animal experimentation but the experiments were repeated thrice in the laboratory to get the confidence of the sero-conversion. The results were also as per the other global reports (Dinter, 1948; Traub, 1947; Opriessnig *et. al.,* 2020) which only claims for such vaccine to be effective for 2-4 months only, similar to our vaccine. Further we could not conduct challenged studies in animals as the required facility is not available with us and need to be carried out in near future. Overall, the vaccine initial results were encouraging and the vaccine protocol is also simple which can be followed by other biological laboratory.

## 4. Conclusion

The formalin killed alum adjuvant *E.rhusiopathiae* vaccine had showed successful results during the limited farm trial and this low cost vaccine technology can be used by vaccine biological/state departments for their in-house use. Even though the disease is sporadic in nature but can cause heavy loss to the pig industry and the application of vaccine can be done where there is recorded prevalence of this disease. Ring vaccination around the outbreak zone also can limit the spread of the disease to a limited geographical area.

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## 6. Conflict of interest-

The authors declare that they have no conflict of interest within themselves and others including the funding agency and the agency where the research was carried out.

## 7. References

Barman N N, Borkotoky D, Borah B, Nath A J, Das P and Borah D P (2016) Seasonal emergence of swine erysipelas in hilly state Nagaland, Northeast India. African Journal of Microbiology Research 10: 2015-20.

Char N L, Rajeswarj K R, Singh K R, Reddy V R, Rao C R and Sreeramulu P (1993) An outbreak of urticarial form of swine erysipelas in Andhra Pradesh. Indian Veterinary Journal 70(1): 1-3.

Das S, Ghatak S, Bhattacharya U, Puro K, Amarjit K, Kumar P K L, Verma D, Ahuja A and Sen A (2014) Identification of *Erysipelothrix rhusiopathiae* infection from a pig farm in Meghalaya, India. Veterinary Practioneer 15(1): 52-54.

Das S, Wahlang L, Khan S, Karam A, Milton AAP *et. al.,* (2020) Sero-prevalence of the neglected zoonoses of porcine erysipelas in the north eastern hill state of Meghalaya, India. Exploratory Animal Medical Research 10(1):60-64.

Dinter Z (1948) Zur Schutzimpfung gegen Schweinerotlauf mit einer Adsorbatvakzine. Tierärztliche Umschau. 3(17-18):279–81.

Kumaresan A, Bajurbaruah KM, Pathak K, Das A and Bordoloi R. (2009). Integrated resource-driven pig production systems in a mountainous area of Northeast India: production practices and pig performance. Tropical Animal Health Production. 41(7):1187-96.

Makino S, Okada Y, Maruyama T, Ishikawa K, Takahashi T, Nakumura M, Ezaki T and Morita H (1994) Direct and rapid detection of *Erysipelothrix rhusiopathiae* DNA in animals by PCR. Journal of Clinical Microbiology 32: 1526–31.

Opriessnig T, Forde T and Shimoji Y (2020) *Erysipelothrix* Spp.: Past, Present, and Future Directions in Vaccine Research. Frontier in Veterinary Science 7:174.

Saini SS, Khera RS, Kwatra MS (1994) An outbreak of acute form of swine erysipelas in Punjab. Indian Journal of Animal Science 64(8): 816-817.

Traub E (1947) Immunisierung gegen Schweinerotlauf mit konzentrierten adsorbat. Impfstoffen. Monatsh. Veterinaermed 2:165–72.