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RESEARCH ARTICLE

Clinical and Economic Impacts of a Commercial Vaccine Containing Recombinant Antigen against Atrophic Rhinitis in Field Conditions

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ABSTRACT

Atrophic rhinitis (AR) is an important disease continuously causing economic losses to pig industry. Due to the detrimental effects on growth performance, antibiotics have been widely used to treat the bacterial diseases in the field. As the concerns about bacterial resistance to antibiotics increase, there is a growing need for the evaluation of AR vaccine that can reflect actual cost and benefits. However, until now, only limited research data have been available. Therefore, in this study, the systemic analyses of clinical and economic benefits by AR vaccination under field conditions were conducted. The trials were performed using a bivalent commercial vaccine containing *Bordetella bronchiseptica* bacterin and recombinant Type D *Pasteurella multocida* toxin in three different commercial pig farms endemic for AR. Significant higher levels of colostral antibodies and those passive transfer were detected from vaccinated sows to their offspring compared to non-vaccinated groups. As predicted on serological data, notably better clinical protection from the deleterious effects of AR was identified in piglets born to the vaccinated sows based on body weight, rearing period, and gross nasal scores. Moreover, vaccination on sows delivered a return on investment at least ten times the cost of vaccination. Collectively, these favorable clinical and economic effects in the commercial farms sufficiently support the necessity and significance of AR vaccination on sows.

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INTRODUCTION

Porcine Atrophic rhinitis (AR) is a contagious upper respiratory disease characterized by nose bleeding, atrophy and distortion of nasal turbinate, and subsequent growth retardation (Dominick and Rimler, 1988, Gwaltney *et al*., 1997; Mullan and Lax, 1998). The disease has been known as to be caused by *Pasteurella multocida* and/or *Bordetella bronchiseptica*. According to disease severity, AR can be classified into two categories; non-progressive and progressive form in affected animals. Whereas non-progressive AR (NPAR) is reversible, progressive AR (PAR) is a specific disease condition described as permanent atrophy of nose tissue (Zimmerman *et al*., 2012).

Bordetella bronchiseptica is one of commensals that colonizes on the porcine respiratory tract and the primary etiological agent of NPAR. Furthermore, the bacterial infection is considered as a predisposing factor of PAR because mucus accumulation and ciliostasis caused by secreted tracheal cytotoxin facilitates colonization of *P. multocida*. Otherwise, PAR can be caused by toxigenic *P. multocida* alone. There are five capsular types of *P. multocida*; A, B, D, E and F. Among them, serotype A is mainly isolated from pneumonic lung and while the most PAR isolates are serotype D. Pm toxin (PMT), the essential factor for the pathogenesis of PAR, induces continuous snout shortening and turbinate atrophy by interfering with remodeling and formation of nasal turbinate bone (Davies *et al*., 2003; Liu *et al*., 2017).

Many types of vaccines against AR have been developed with various combinations of whole cell bacterins and inactivated or recombinant forms of PMT (Liao *et al*., 2006; Kang *et al*., 2008; Hsuan *et al*., 2009; Gu *et al*., 2017). Among them, vaccine containing antigens from both pathogens is regarded as most effective based on the following facts; (1) Monovalent PMT vaccine showed inferior efficacy compared to

bivalent *B. bronchiseptica* and *P. multocida* vaccine at challenge experiment. (2) Monovalent *B. bronchiseptica* bacterin vaccine gives no protection against toxigenic *P. multocida*, which cause considerable variation in the field test result (Magyar and Lax, 2002).

AR in pigs is usually accompanied by low weight gain (Scheidt *et al*., 1990; Donko *et al*., 2005) and increases mortality when complicated with porcine respiratory disease complex in commercial farms (Choi *et al*., 2003), which leads to significant economic losses of swine industry. However, until now, the economic impact of sow vaccination against AR has not been well examined systemically in previous studies. Moreover, the need for such estimates becomes even more apparent as the interest in bacterial vaccines grows with the recognition of problems with antibiotic resistance (Furian *et al*., 2016; Yeh *et al*., 2017). The purpose of this study is to determine the clinical and economic impact of a commercial bivalent vaccine (RHINISENG®, Hipra, Spain) composed of *B. bronchiseptica* bacterin and recombinant PMT under field condition. The investigation was performed in the three AR endemic commercial farrow-to-finish pig farms and the assessment factors included antibody titers against *Bordetella bronchiseptica* and *P. multocida*, antigen detection rate, gross nasal scores, body weights, time required for pigs to reach market weight, and return on investment (ROI).

MATERIALS AND METHODS

Vaccine: RHINISENG® (HIPRA, Spain), an inactivated vaccine containing *B. bronchiseptica* bacterin (Bb) and recombinant Type D PMT, was used. Sows received double injection of 2 ml RHINISENG® intramuscularly 6 weeks and 3 weeks before the expected parturition date. Control animals received 2ml of phosphate buffered saline.

Experimental schedule: Three different conventional pig farms endemic for AR (farm A, B, and C) with 200, 150, and 110 sows, respectively, were chosen through preliminary studies on farm histories, serum antibody titers, antigen detection rate, and gross nasal scores.

All experiments were performed according to the registration guidelines of Animal, Plant & Fisheries Quarantine & Inspection Agency (http://qia.go.kr). Twenty sows per each farm were selected and randomly divided into vaccinated (n=10) and control group (n=10). Vaccination influence was estimated in sows and their offspring based on clinical, serological, and economic parameters. Antibody titers against PMT and Bb were determined per pig farm in colostrum (n=10; 5 of vaccinated sows and 5 of nonvaccinated sows) and serum samples (n=40; 20 from vaccinated sows and 20 from nonvaccinated sows) of 5-day-old and 6-week-old piglets. Antigen detection of *P. multocida* type D and *B. bronchiseptica* on nasal swabs was performed with measuring gross nasal scores (n=40; 20 from vaccinated sows and 20 from non-vaccinated sows) of 6-week-old piglets and pigs of slaughter age. The nasal score was measured using previously described method (Magyar *et al*., 2002). Body weight and a total fattening period required for piglets to reach market weight were also assessed in 6-week-old piglets and pigs of slaughter age with the calculation of return on investment (ROI).

Clinical, serological and economic assessment

Body weights and fattening periods with ROI calculation: Live body weights of 6-week-old piglets (n=40; 20 from vaccinated sows and 20 from non-vaccinated sows) and carcass weight of pigs at slaughterhouse (n=40; 20 from vaccinated sows and 20 from non-vaccinated sows) were measured. Because the finishers are reared until their weight reach around 110kg, feeding period was used as an estimation factor. Further, return on investment (ROI) during the investigation period was calculated for numerical evaluation of vaccine impact as follows: (Differences in income over feed costs between experimental and control group) / (Vaccination cost). Income over feed costs (IOFC) was obtained by subtracting feed costs from sales income by selling pigs at the market weight. For animals removed due to natural death or intentional euthanasia for animal welfare, feed costs during the life of animals were considered for ROI calculation. All the estimated costs were calculated in Korean won (KRW) and converted into US dollars (USD) at a rate of KRW 1,126=US$1.

Antigen and antibody detection: PCR analysis was performed as described by Townsend *et al*. (2001) for *P. multocida* type D detection and by Hozbor *et al*. (1999) for *B. bronchiseptica*. Microagglutination test (MAT) and ELISA were implemented for antibody titration against Bb and PMT, respectively. MAT test was performed using antigen (Bb) obtained from CIVTEST (Spain) and the presence of antibodies against *P. multocida* toxin D was determined by a blocking PMT ELISA (Oxoid, Hamshire, UK).

Statistical analysis: SPSS ver. 21 (SPSS Inc., Chicago, USA) was used for statistical analysis. Differences in bacterial detection rate between groups were evaluated by Fisher`s exact test. Correlation between the antigen detection rate and the severity of nasal atrophy was analyzed by linear and linear association method. For the comparison of antibody titers, body weight, rearing period, nasal scores, and ROI between groups, student t-test and Mann-Whitney test were selectively used according to the group normality. Statistical significance was set as a P value under 0.05.

RESULTS

Titers of antibody against PMT and *B. bronchiseptica*

Sows (colostrum): Colostral antibody titers against PMT and *B. bronchiseptica* of experimental group showed higher value than that of control group with statistically significant difference ($P<0.05$) in all three different farms (Fig. 1). The percentages of PMT ELISA positive specimens from farm A, B, and C were 100, 80, and 90% in experimental group and 60, 0 and 50% in control group, respectively.

Piglets (Serum): In all three experimental farms, the mean antibody titers against PMT and *B. bronchiseptica* in serum samples collected from 5-day-old and 42-day-old piglets of experimental group was superior to those of control groups and the difference was statistically significant ($P<0.05$) (Fig. 2).

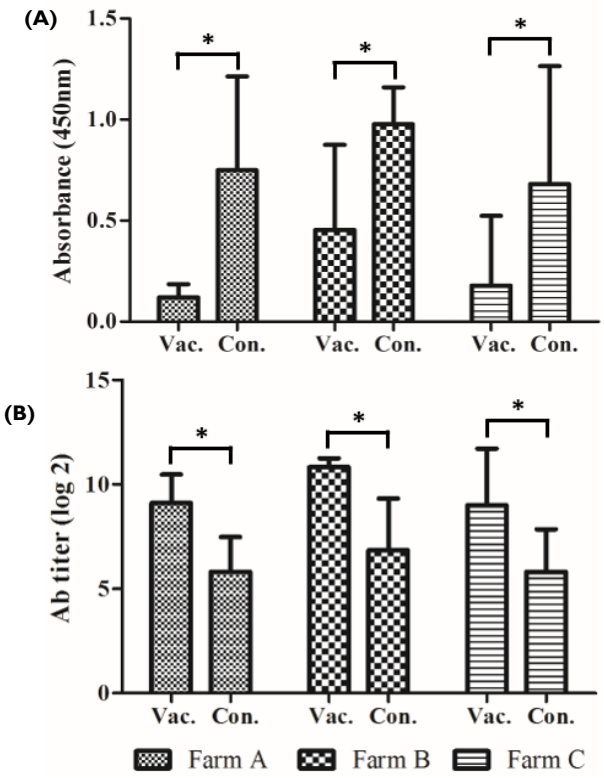

Fig. 1: The colostral antibody titers of sows against PMT (A) and *B. bronchiseptica* (B) in three different farms between groups. In figure 1A, the lower absorbance means the higher antibody titers since the PMT ELISA applied in this study is blocking type. Statistical results were considered to be significant when P values were lower than 0.05 (*).

Table 1: Antigen detection rate on nasal turbinate swabs of 6-week-old pigs and finishers at slaughter age in three pig farms

Farm	Sow group	Offspring group	Antigen detection rate (%)	
			P. multocida type D (%)	*B. bronchiseptica* (%)
A	Vaccinated	6-week-old	40 (8/20)	15 (3/20)
		Slaughter age	20 (4/20)	25 (5/20)
	Control	6-week-old	50 (10/20)	30 (6/20)
		Slaughter age	45 (9/20)	50 (10/20)
B	Vaccinated	6-week-old	10 (2/20)	15 (3/20)
		Slaughter age	05 (1/20)	15 (3/20)
	Control	6-week-old	15 (3/20)	20 (4/20)
		Slaughter age	10 (2/20)	10 (2/20)
C	Vaccinated	6-week-old	00 (0/20)	05 (1/20)
		Slaughter age	05 (1/20)	25 (5/20)
	Control	6-week-old	10 (2/20)	15 (3/20)
		Slaughter age	15 (3/20)	20 (4/20)

Antigen detection rate

Six-week-old piglets: Relatively low detection rate of both *P.multocida* type D and *B. bronchiseptica* in 6-wk-old pigs of experimental group compared to control group was detected in all three farms (Table 1). However, there was no statistical difference between vaccinated and control group. Similar to the preliminary test, the detection rate of *P. multocida* type D increased significantly in accordance with the severity of nasal atrophy ($P<0.05$), while that of *B. bronchiseptica* had no correlation with nasal scores (data not shown).

Finishers: In all experimental groups of three pig farms, *P. multocida* type D was detected less frequently when compared to control group. However, there was no tendency for *B. bronchiseptica* to be detected in a lower rate in control group (Table 1).

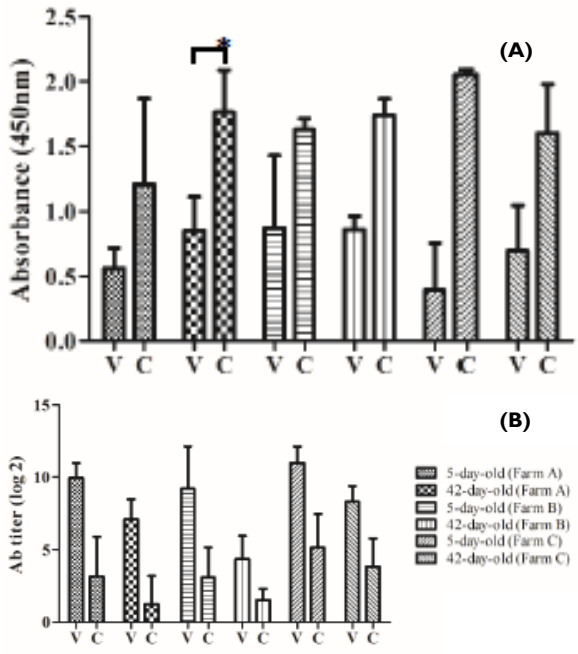

Fig. 2: The serum antibody titers of 5-day-old and 42-day-old piglets against PMT (A) and *B. bronchiseptica* (B) in three different farms between groups. In figure 2A, the lower absorbance means the higher antibody titers since the PMT ELISA applied in this study is blocking type. Statistical results were considered to be significant when P values were lower than 0.05 (*).

Table 2: Economic evaluation of sow vaccination against AR in three experimental pig farms

Parameters	Sow group	Experimental farms		
		Farm A	Farm B	Farm C
Marketed pigs (n)	Vaccinated	103	96	98
	Control	98	96	98
Total feed costs ($)	Vaccinated	12,238	12,158	11,955
	Control	12,532	13,153	12,839
Total Sales ($)	Vaccinated	29,538	34,691	33,064
	Control	28,626	33,923	32,629
IOFC ($)	Vaccinated	17,300	22,533	21,109
	Control	16,094	20,770	19,790
ROI		14.2	20.7	15.5

No statistical significance was identified in detection rate of *P. multocida* type D and *B. bronchiseptica* between groups. As similar to 6-week-old pigs, finishers of three pig farms exhibited significant positive correlation between the detection rate of *P. multocida* type D and nasal lesion severity ($P<0.05$). On the contrary, the correlation was not shown in *B. bronchiseptica* (data not shown).

Mean Body weight, fattening period and ROI

Six-week-old piglets: Mean body weight of 6-week-old piglets of experimental and control group of farm A were 9.4±1.51 kg and 7.5±2.04 kg, respectively. Those of farm B and C were 9.9±1.44 kg, 7.6±2.33 kg and 8.7±1.41 kg, 7.7±2.14 kg, respectively (Fig. 3A). The difference between two groups was statistically significant in all three different farms ($P<0.05$).

Finishers: At the time of slaughter, the carcass weight of vaccinated and control group was 88.9 kg±0.85, 87.0 kg±0.87, respectively. Those of farm B and C were 85.6 kg±7.14, 84.8 kg±7.40 and 84.7 kg±1.84, 85.8 kg±1.74, respectively. There was no statistically significant difference

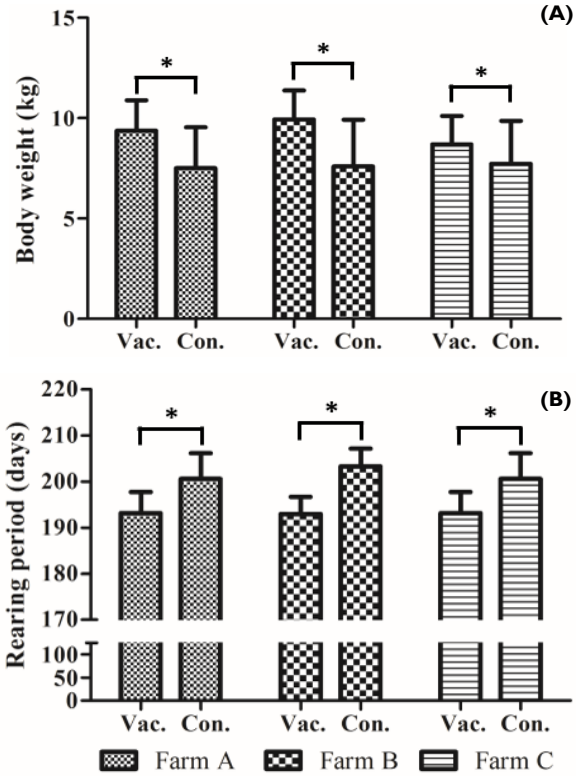

Fig. 3: The body weight of 6-week-old piglets (A) and the rearing period of (B) between groups in three different farms. Because there were no statistical differences in carcass weight, total rearing period were compared between groups. Statistical results were considered to be significant when P values were lower than 0.05 (*).

Table 3: Mean turbinate conchal score of 6-week-old and slaughter age offspring piglets

Farm	Sow group	Gross nasal score[a]	
		6-week-old[b]	Slaughter age[b]
A	Vaccinated	3.0	4.8
	Control	6.5	6.2
B	Vaccinated	2.6	4.4
	Control	4.9	6.6
C	Vaccinated	2.0	4.1
	Control	4.9	5.4

[a]The score ranges of gross nasal lesions were from 0 (normal) to 18 (complete atrophy); [b]The mean nasal score between experimental and control groups differ significantly (P<0.05).

between two groups of farm A, B, and C. However, the average marketing age of experimental group (farm A) was about 8 days younger than that of control group. Similarly, the feeding period of the vaccine group of farms B and C was shorter than that of the control group by about 10 days (Fig. 3B). The difference in rearing period between groups were statistically significant in all three farms (P<0.05). Considering IOFC and vaccination costs (4.26 USD per dose), it was calculated that ROI of sow vaccination with RHINISENG® of farm A, B, and C were 14.2, 20.7, and 15.5, respectively (Table 2).

Nasal scores

Six-week-old piglets: The average nasal scores of vaccinated and control groups of farm A were 3 and 6.5 and those of farm B and C were 2.6, 4.9 and 1.95, 4.9, respectively (Table 3). There was statistically significant difference in nasal scores of 6-week-old piglets between experimental and control groups (P<0.05).

Finishers: The mean nasal score of finishers of experimental group and control group in farm A was 4.8, 6.2, respectively. Those of farm B and C were 4.5, 6.6 and 4.1, 5.4, respectively (Table 3and Fig. 4). There was statistically significant difference in nasal scores of pigs of slaughter age between two groups (P<0.05).

DISCUSSION

Atrophic rhinitis is an economically important disease for pig producers because affected farms are characterized by prolonged feeding period and relatively high mortality rate of finisher herds (Wilson and Ho, 2013; Lopes Antunes *et al*., 2017). While the single pathological effect of *B. bronchiseptica* and *P. multocida* Type D on weight gain has not been clearly demonstrated, the combined infection of the two bacteria is likely to be responsible for decreased growth rate (van Diemen *et al*., 1994; Magyar *et al*., 2002; Wilson and Ho, 2013). In this study, the influences of sow vaccination against AR using RHINISENG® (HIPRA, Spain) were analyzed under field conditions from clinical and economic perspectives.

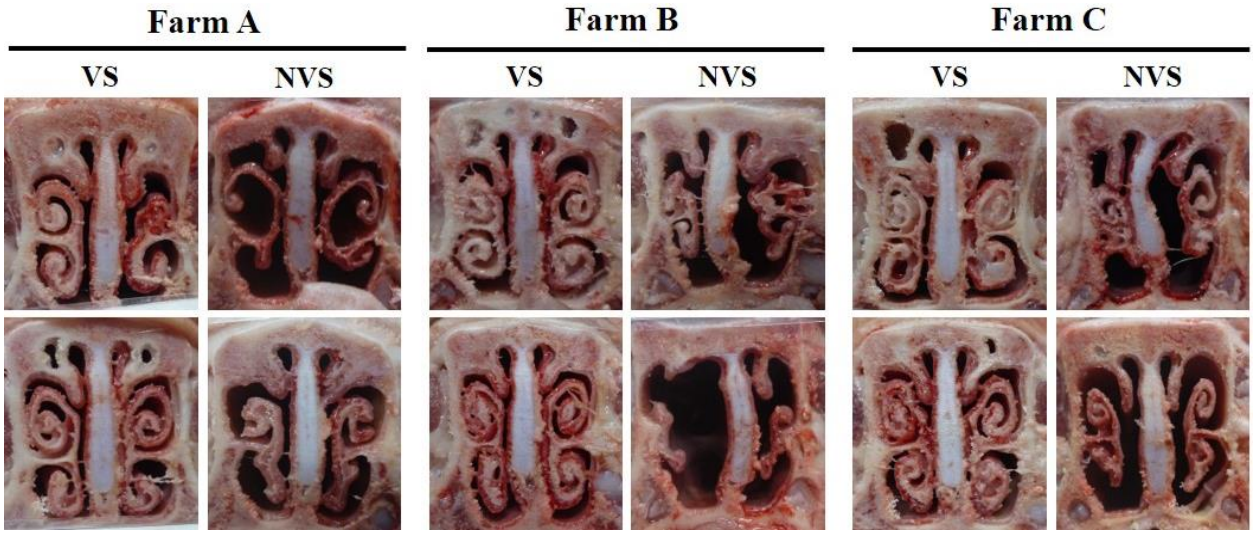

Fig. 4: Representative images of nasal section of pigs at slaughter age from vaccinated sows (VS) and nonvaccinated sows (NVS) per experimental farm A, B, and C.

It was identified that the vaccine RHINISENG® offered the significant clinical and economic benefits based on following findings; Effective transfer of maternal antibody against PMT and *B. bronchiseptica* to suckling piglets. High protective efficacy against AR in 6-week-old and pigs of slaughter age based on nasal lesion scores, body weight, and total feeding period. ROI of three pig farms were 14.2, 20.7, and 15.5 when the vaccination against AR was implemented on sows. Although the estimates varied between farms according to farming conditions, marketing time, and market price of finishing pigs, AR vaccinated sows delivered economic profits more than ten times the cost of vaccination over the non-vaccinated sows. To the best of our knowledge, this is the first investigation to analyze the economic impact of vaccine use against AR on pig industry.

It was identified that vaccination did not make significant influences on antigen detection rate of both microorganisms in this study. Interestingly, other studies also reported similar result that vaccination produced no differences in the prevalence of *B. bronhiseptica* infection under both natural and experimental conditions (Magyar and Lax, 2002). Considering that *P. multocida* itself poorly colonizes the nasal cavity without *B. bronhiseptica* (Horiguchi, 2012) and the vaccine used in this study stimulates the immunity against not bacteria itself but PMT, it can be partially explained that no significant difference in the detection rate of *P. multocida* between experimental and control groups was observed.

It is known that nontoxigenic *P. multocida* type D has relatively low relevance with turbinate atrophy (Ackermann *et al*., 1994; Magyar *et al*., 2013). A limitation of the current study was the absence of differentiation process between toxigenic and non-toxigenic strains of *P. multocida* type D. However, the correlation between the antigen detection rate (*P. multocida* type D; positive correlation and *B. bronchiseptica;* no significant correlation) and nasal lesion scores and significantly improved brachygnathia after vaccination in all three experimental farms indirectly reflects toxigenic clinical influence of *P. multocida* type D. Colostrum samples of non-vaccinated sows of farm B showed 0% of positive rate of PMT antibodies, which is the quite dissimilar results from farm A and C. This contradictory result could be attributed to the following facts; (i) Prior use of vaccine with an antigenic composition similar with RHINISENG® has been identified in farm A and C but not in farm B (ii) Natural PMT is regarded as poor immunogen as described by others; PMT constitutes less than 0.6% of total cellular proteins (Liao *et al*., 2006.; Hsuan *et al*., 2009).

Conclusions: Vaccination with a commercial bivalent vaccine RHINISENG® on sows effectively conferred protective immunity against AR to their offspring with providing substantial economic benefits to pig producers. Therefore, these findings highlight the importance of AR vaccination on sows for successful pig production.

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Authors contribution: YSL conceived and designed this study. MHK, SJY, TYK, SHJ, DUL, JJB and JYS executed the experiment. MHK, SJY and TYK participated in the interpretation of data and drafting the manuscript. YSL and SWS contributed to critical revision of the paper. All authors read and approved the final manuscript.

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