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Standardization of Pro-Inflammatory Cytokines (IL-1 & TNF-α) and Haematological Biomarker as an Early Assessment of Health Status in Adult Healthy Pigs

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Abstract

The study was aimed to standardize pro-inflammatory cytokines and haematological biomarker level in visually adult healthy swine as an early health check-up scanner. In the present study, total six (6) crossbreed pigs (Yorkshire × Swedish Landrace) with similar age group (3 years) and unisex (male) with average body wt (140 kg) were selected for sampling. Analyzed serum samples revealed that, average values of pro-inflammatory cytokines TNF-α was 242.33± 10.23 ng/ l and IL-1 was (12.16±1.16 ng/l). After analyzed, haematological biomarker showed that, average values of TEC was 6.43±0.14 10 6/µl, Hb was 12.33±0.33 gm/dl, HCT was 37.68±0.5%, PCV was 36.5±1.1%, MCV was 52.66±1.1, MCH was 16±0.8fl, MCHC was 30.83±0.71pg, TLC was 14.75±0.61/µl, neutrophils was 37.2±0.89%, lymphocyte was 54.6±0.62%, monocyte was 2.16±0.34%, eosinophils was 4.86±0.18%, basophils was 1.16±0.18%, platelets was 4.49±0.1 105/µl and total protein was 6.57±0.23 g/dl. In the statistical analysis positive correlation was found between pro-inflammatory cytokines (TNF-α & IL-1β) and routine haematological inflammatory indicators mainly WBC and neutrophils. Lymphocyte showed negative correlation with pro-inflammatory cytokines and other haematological biomarker.

Keywords: Pro-inflammatory cytokines; Indicator; Immune; Poor hygiene; Pig health

Introduction

Sus scrofa domesticus is homoeothermic animal that have the ability to maintain core body temperature. Extreme fluctuating weather is a major challenge to maintain core body temperature and immune status. In the subtropical climatic condition of India, pigs are mainly habitat under commercial or natural condition in India. However, surrounding hygienic status is major hazard for health status and wellbeing of animals. Poor hygiene level, continuously predisposed to animal for interact to various pathogenic and non-pathogenic micro-organisms throughout entire life. Besides this, some micro-organisms do not clinically affect but they have the ability to produced proinflammatory cytokines and disarrangement of haematological dynamic. These exposures typically respond to their immunological response system (cellular and humeral components) by stimulate the release of pro-inflammatory cytokines and rearrangements of haematological biomarkers (Kim, 2012) [1]. Cytokines such as tumor necrosis factor (TNF-α) is produced by activated macrophages, monocytes, and various other cells. TNF-α is more closely associated with proinflammatory effects (Silva *et al.* 2019) [2] against antigenic challenges. Interleukin-1 is a key cytokine that produces by many porcine cells such as macrophages and intestinal epithelial cells (Standyk, 2002) [3]. It is well known to influence thymocyte proliferation and B-cell growth and differentiation. It also needed for the production of, interleukin-2, interleukin-6 and chemokine attractions for neutrophils and interleukin-8 (Armstrong *et al.,* 2004). IL-1 not only response during infection but it also affected by appetite, sleep and body temperature (Allen et al. 2005 and Dinarello et al. 2012) [4,5] so it can be used for monitoring the status of animal welfare and health. Increased proinflammatory cytokines generally have also a negative influence on growth and well-being (Stahly *et al.*1996) [6]. TNF-α is usually produced simultaneously with interleukins and potentializing pro-inflammatory vascular and cellular alterations (Meyer et al. 2003) [7]. Therefore, TNF-α and IL-1 are usually produced together as a prompt response to bacterial infections. Together they both modulate pro-inflammatory signals by coordinating vascular and cellular changes in the immune system, activating many of the components present in the innate immunity, increasing the expression of chemokine and adhesion molecules, critical for the recruitment of neutrophils from the blood, initiating the immune first lineage prompt response in the host (Bazzoni et al.1996, Dinarello *et al.* 1997 and Mehrad *et al.* 1999) [8-10]. As inflammation progresses, leukocyte, lymphocyte, and other cells are activated and recruited at inflamed site by a signalling network. Haematological biomarker are key indicators for health and disease surveillance in all animals as well as swine (Mbanasor et al. 2003). Subnormal inflammatory chain reaction in the body generates more free radical which responsible for further adverse effects on health, production, and body weight gain. The importance of pro-inflammatory cytokines (TNF-α and IL-1) and routine a haematological biomarker has kept in mind, study was designed to evaluate the selected parameters, it might be helpful for surveillance of swine health and wellbeing.

Materials and Methods

Selection of animals

Six (6) crossbreed pigs (Yorkshire × Swedish Landrace) were selected with a similar age group (3 years), unisex (male), and 140 kg average body wt for the evaluation of and pro-inflammatory cytokines and haematological indicators values. All the selected pigs had healthy body conditions, normal skin colour, and visually normal physiological activity (Figure 1).

Figure 1: Crossbred pig (Yorkshire × Swedish Landrace)

Samples collection

The pig was restrained and bled through the caudal vena cava as described by (Carle and Dewhirst, 1942) into a blood collection tube containing ethylene-diamine tetra-acetic acid (EDTA) as an anticoagulant for hematological study and for serum study blood was collected in plain blood collection viol.

Estimation of routine hematological biomarker

The blood samples were analyzed for total erythrocyte count (TEC), haemoglobin concentration (Hb), packed cell volume (PCV), and total and differential leukocytes count. The PCV was determined using the microhaematocrit method while the RBC and leukocyte counts were determined using the improved Neubauer counting chamber (Jain, 1986) [11]. Smears for differential leukocyte counts were stained by the Leishman technique, and the different cells of leukocyte series were enumerated by the longitudinal counting method. The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated from the PCV, Hb, and RBC count.

Cytokines bioassays

Serum concentrations of interleukin-1 and tumor necrosis factor -α were determined with commercially available ELISA kits for detection of porcine cytokines by using the described protocol by (Chongqing Biopes, Co, Ltd. China). Cytokines assay (TNF and IL-1), were evaluated in each well of ELISA plate at last step addition of kit component 7 and finally mixing thoroughly, it produced immediately color changes into yellow that showed (Figure 2). After the appearance of yellow color noted the ELISA plate reading and calculated the average mean values of 6 samples.

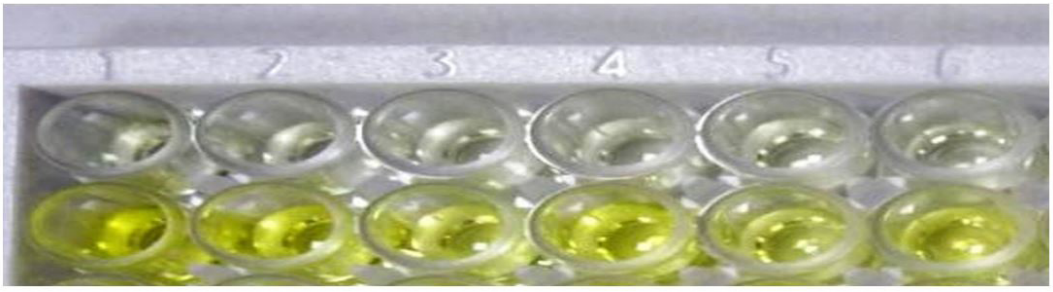


Figure 2: Final colour developments in ELSA Plate for Porcine TNF α

Statistical analysis

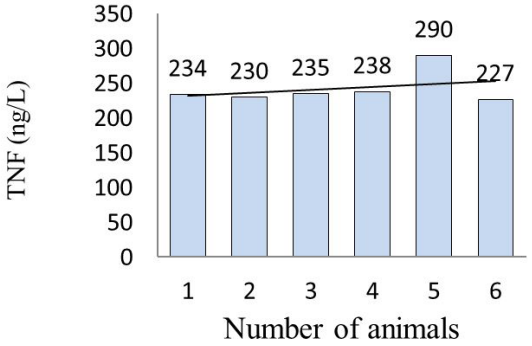
Descriptive analysis is presented as individual value, group mean and standard error. Pearson's correlation coefficient was calculated for the relationship between all qualitative variables by using Excel Micro software.

Results

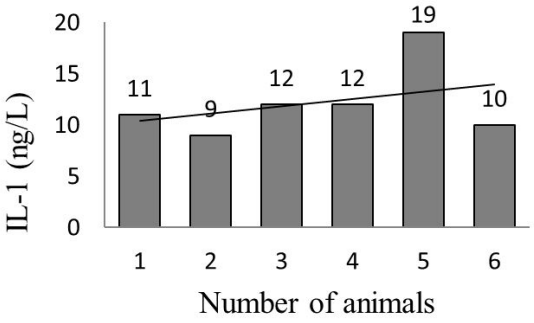
Mean and individual serum values of cytokines of adult porcine are presented in Table 1 and Graph A and B. The mean cytokines values (n=6) of TNF-α was 242.33± 10.23 ng/l and IL-1 was12.16±1.16. The mean values of cytokines were statistically non-significant with individual value as well as reference values. However, the highest value of TNF-α and IL-1 was measured to be 290.0 ng/l and 19.0 ng/l while the lowest was 230.0 ng/l and 9 ng/l respectively.

S.N	Assay	Unit	Animals						Group Mean	Reference
			1	2	3	4	5	6		
1-	TNF-α	ng/L	234	230	235	238	290	227	242.33± 10.23	Liu et al., 2010
2-	IL-1	ng/L	11	9	12	12	19	10	12.16±1.16	

Table 1: Analysis of mean values of cytokines in serum of adult porcine



Graph A: TNF value of individual porcine

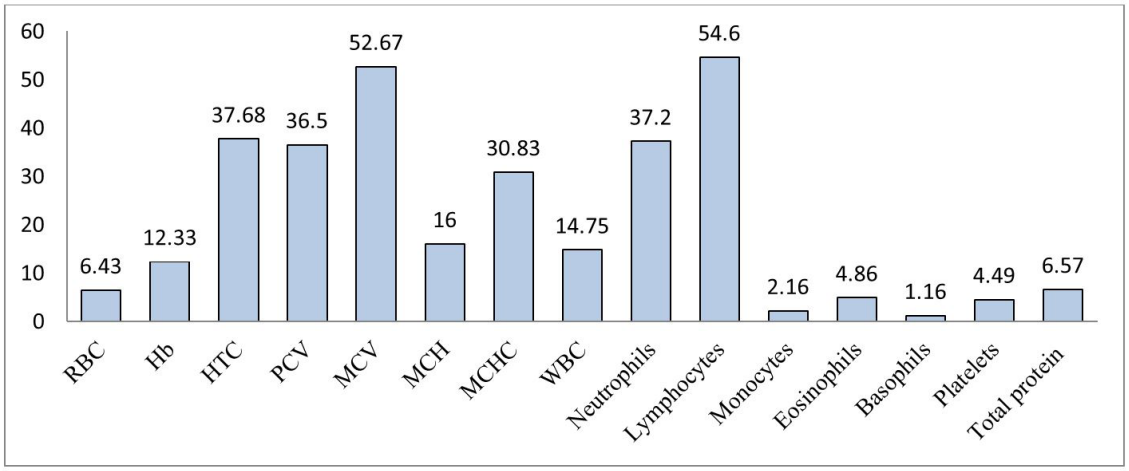


Graph B: IL-1value of individual porcine

S.No	Parameters	Unit	Mean (n=6)	Min	Max	References
1.	RBC	10^6/µl	6.43±0.14	6.36	6.80	5.0 – 8.0
2.	Hb	gm/dl	12.33±0.33	10	14	10.0 – 16.0
3.	HTC	%	37.68±0.5	33.6	40	28.3 - 42.7
4.	PCV	%	36.5±1.1	28	47	32 – 50
5.	MCV	fl	52.66±1.1	47	62	50 – 68
6.	MCH	pgm	16±0.8	13	20	17.0 – 21
7.	MCHC	gm/dl	30.83±0.71	28	34	30.0 – 34.0
8.	WBC	10^3/µl	14.75±0.61	9.00	20.0	11.00 – 22.00
9.	Neutrophils	%	37.2±0.89	34	40	28 – 47
10.	Lymphocytes	%	54.6±0.62	50	60	39 – 62
11.	Monocytes	%	2.16±0.34	1	3	2 – 10
12.	Eosinophils	%	4.86±0.18	3	7	0.5 – 11
13.	Basophils	%	1.16±0.18	0	2	0-2
14.	Platelets	10^5/µl	4.49±0.1	3.57	5.12	5.2 ± 1.95
15.	Total protein	g/dl	6.57±0.23	5.68	7.6	7.0 – 8.0

Table 2: Analysis of mean hematologic parameters of adult porcine (Schalm, 2000)

Statistical analysis of haematological indicators values are presented in Table 2 and Graph C and it showed that, mean red blood cell count was 6.43±0.14 10 ^{6}/µL, mean haemoglobin concentration was 12.33±0.33g/dl and mean PCV was 36.5±1.1%. RBC indices were also ranges with reference values in growing porcine. Mean count of WBC was showed moderate increment under reference range. The percentage of neutrophils was found to be lower but the percentage of lymphocyte and eosinophils were observed comparatively higher than references values of other domestic animals respectively.

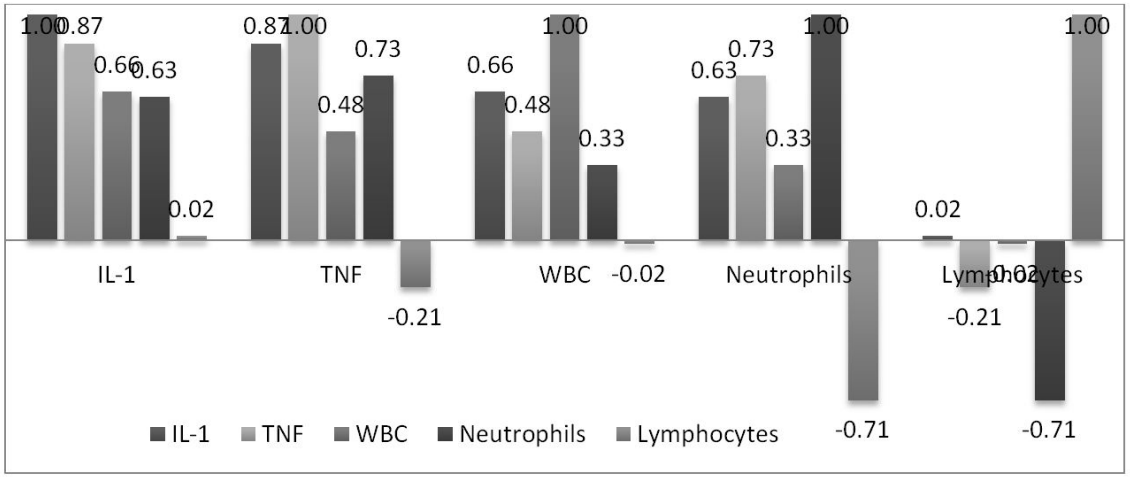


Graph C: Mean of hematologic parameters of Adult porcine

Correlation level of pro-inflammatory cytokines and routine haematological biomarker are present in Table 3 and Graph D. Correlation coefficient values showed that, positive correlation between IL-1 and other three variables TNF-α, WBC and neutrophils except lymphocytes that was showed very poor correlation with IL-1 as well as negative correlation with TNF-α (-0.21), WBC (-0.02) and neutrophils (-0.71).

Parameters	IL-1	TNF-α	WBC	Neutrophils	Lymphocytes
IL-1	**1.00**	0.87	0.66	0.63	0.02
TNF	0.87	1.00	0.48	0.73	-0.21
WBC	0.66	0.48	1.00	0.33	-0.02
Neutrophils	0.63	0.73	0.33	1.00	-0.71
Lymphocytes	0.02	-0.21	-0.02	-0.71	1.00

Table 3: Correlation level of pro-inflammatory cytokines and routine haematological inflammatory indicator



Graph D: Correlation levels of pro-inflammatory cytokines and routine haematological inflammatory indicator

Discussion

Pro-inflammatory cytokines and routine haematological parameters are reliable indicators for the assessment of animal health and wellbeing (Wolmarans *et al.,* 2011) [12]. Cytokines bioassay of TNF-α and IL-1 revealed that the concentration of both the cytokines was similar to findings of previous studies. (Beisel *et al.*,1991 and Stahly *et al*., 1996) [6,13]. Serum concentration of proinflammatory cytokines are directly influenced by what the animal exposed to be, it viruses, intracellular or extracellular bacteria, myco-toxin or non-infectious damage (Nordgreen *et al.,* 2020) [14]. Unhygienic environments are typically dusty and exposure to dust triggers a cytokine response (Sahlander *et al.,* 2012) [15]. Gaseous component of air like ammonia causes the releases of cytokines by alveolar macrophages and neutrophils (Murata *et al.,* 1999) [16]. Psychological stress one of the most important inducing factors of proinflammatory cytokine production in humans as well as rodent (Maes *et al.,* 1998) [17]. In the pig, psychological stress might affect the immune response (Manciocco *et al.,* 2011) [18]. Protein deficient diet particularly tryptophan under low sanitary the condition can influence the cytokine production in pig (Nordgreen *et al.,* 2020) [14]. Release of cytokines activated the cellular and humoral components of the immune system such as phagocytes and antibodies. The active immune system of the body is singling the rearrangement dynamics of perivascular blood components.

In the statistical analysis haematological biomarker of health status showed that non-significant differences between lower and higher mean values of total erythrocyte count, haemoglobin, HCT, MCH, MCHC, and MCV. It means, the health status of swine could be varying in terms of minerals, vitamins levels oxygen-carrying capacity of the blood, and hydration status of the body. A significant difference was found between lower and higher mean values of total leukocyte counts. It might be due to the different immune status of individuals. Acute tissue damage or bacterial invasion in the body generates a wide variety of signals for the early recruitment of neutrophils (De *et al.,* 2016) [19] lead to excessive migration of neutrophils towards the peripheral circulation. Lymphocytes usually respond to foreign antigens only if the innate immune system is first activated (Alberts *et al.,* 2002) [20]. Clinical and subclinical viral infection enhances lymphocyte proliferation, expressed in the peripheral circulation. Monocytes are recruited to infected tissues and mediate direct antimicrobial activity at this site (Serbina *et al.,* 2008) [21]. Basophils are the least abundant leucocytes primarily found in the circulation, but rapidly expand in the bone marrow in response to inflammatory signals and are mobilized to the blood, spleen, lung, and liver (Min, *et al.,* 2012) [22]. Eosinophils have classically been associated with host defense against parasitic infections, particularly caused by helminths (*Taenia solium*) in swine (Ramirez *et al.,* 2018) [23].

Correlation coefficient, statistical measured for analyzed the strength of the relationship between selected parameters (Table 3 and Graph D) [24]. IL-1 were more positively correlated with TNF-α (r=0.87), WBC (r=0.66), Neutrophils (r=0.63), and non-significantly positive with Lymphocytes(r=0.02).It indicates that initial active immune responses positively triggered the TNF-α, WBC, and Neutrophils' action. TNF-α were also showed significantly positive correlation with IL-1 (r=0.87), WBC (r=0.48), Neutrophils (r=0.73), and non-significantly negative with Lymphocytes(r=-0.21). It means that initial active immune responses positively triggered the TNF-α, WBC, and Neutrophils' action but negatively with lymphocytes because activation is a delayed process.WBC positively correlated with all the variables except lymphocyte. The relationship between Neutrophils and lymphocytes were showed significantly negative. The relationship of lymphocyte with other variables showed negative except IL-1.

Conclusion

The study concluded that each value of the immunological parameter and haematological biomarker was found to be within normal reference range while at individual-level some variation showed because of seasonal and nutritional variation can be considerable. Particularly, immunological TNF-α is more closely associated with proinflammatory effects and IL-1 was originally named "lymphocyte activating factor" because it stimulates lymphocyte proliferation in the presence of suboptimal doses of mutagen. On the basis of the above finding, it can be concluded that pro-inflammatory cytokines and haematological biomarker is good and reliable parameters for assessment of health status and welfare in the pig industries of India.

References

1. Kim KH, Lee EN, Park JK, Lee JR, Kim JH, et al. (2012) Curcumin attenuates TNF-α-induced expression of intercellular adhesion molecule-1, vascular cell adhesion molecule-1 and proinflammatory cytokines in human endometriotic stromal cells. Phytotherapy Res 26: 1037-47.

2. Silva LB, Dos SN, Maia SM, Dos SG, Quidute IL, et al. (2019) The Role of TNF-α as a Proinflammatory Cytokine in Pathological Processes. The Open Dent J 13.

3. Stadnyk AW (2002) Intestinal epithelial cells as a source of inflammatory cytokines and chemokines. Can J Gastroenterol 16: 241-6.

4. Allen SM, Tyrrell PJ, Rothwell NJ (2005) Interleukin-1 and neuronal injury. Nature Rev Immunol 5: 629-40.

5. Dinarello CA, Simon A, Van DMJW (2012) Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases. Nature Rev Drug Discov 11: 633-52.

6. Stahly T (1996) Impact of immune system activation on growth and optimal dietary regimens of pigs: 197- in Recent Advances in Animal Nutrition, Nottingham Univ Press, Nottingham, UK.

7. Meyer O (2003) Role of TNF-alpha and cytokines in the physiopathology of rheumatoid arthritis. Therapeutic perspectives. Bulletin de Academie nationale de medecine 187: 935-54.

8. Bazzoni F, Beutler B (1996) The tumor necrosis factor ligand and receptor families. N Engl J Med 334: 1717-25.

9. Dinarello CA (1997) Interleukin-1. Cytokine Growth Factor Rev 8: 253-65.

10. Mehrad B, Standiford TJ (1999) Role of cytokines in pulmonary antimicrobial host defense. Immunol Res 20: 15-27.

11. Jain NC (1986) Schalm's Veterinary Hematology (4th Edn) Philadelphia: Lea and Febig, USA.

12. Wolmarans WJ (2011) The effect of transport on live weight loss, meat quality and blood haematology in slaughter ostriches: master's thesis Stellenbosch, ZA: University of Stellenbosch, South Africa.

13. Beisel WR (1991) Nutrition and Infection In: Nutritional biochemistry and metabolism with clinical application (2nd Edn) M. C. Linder, USA.

14. Nordgreen J, Edwards SA, Boyle LA, Bolhuis JE, Veit C, et al. (2020) A Proposed Role for Pro-Inflammatory Cytokines in Damaging Behavior in Pigs. Front Vet Sci 7: 646.

15. Sahlander K, Larsson K, Palmberg L (2012) Daily exposure to dust alters innate immunity. PLoS One 7: e31646.

16. Murata H, Horino R (1999) Effects of in vitro atmospheric ammonia exposure on recovery rate and luminol-dependent chemiluminescence of bovine neutrophils and bronchoalveolar macrophages. J Vet Med Sci 61: 279-81.

17. Maes M, Song C, Lin A, De Jongh R, Van Gastel A, et al. (1998) The effects os psychological stress on humans: increased production of pro-inflammatory cytokines and a TH1-like response in stress-induced anxiety. Cytokine 10: 313-8.

18. Manciocco A, Sensi M, Moscati L, Battistacci L, Laviola G, et al. (2011) Longitudinal effects of environmental enrichment on behaviour and physiology of pigs reared on an intensive-stock farm. Italy J Anim Sci 10: 9 10.4081.

19. De OS, Rosowski EE, Huttenlocher A (2016) Neutrophil migration in infection and wound repair: going forward in reverse. Nature Rev Immunol 16: 378.

20. Alberts B, Johnson A, Lewis J, Raff M, Roberts K (2002) Lymphocytes and the cellular basis of adaptive immunity In: Molecular Biology of the Cell (4th Edn) Garland Science, USA.

21. Serbina NV, Jia T, Hohl TM, Pamer EG (2008) Monocyte-mediated defense against microbial pathogens. Annu Rev Immunol 26: 421-52.

22. Min B, Brown MA, Le GG (2012) Understanding the roles of basophils: breaking dawn. Immunol 135: 192-7.

23. Ramirez GA, Yacoub MR, Ripa M, Mannina D, Cariddi A, et al. (2018) Eosinophils from physiology to disease: a comprehensive review. BioMed Res Int doi.org/10.1155/2018/9095275 .

24. Liu Y, Shi W, Zhou E, Wang S, Hu S, et al. (2010) Dynamic changes in inflammatory cytokines in pigs infected with highly pathogenic porcine reproductive and respiratory syndrome virus. Clin Vacc Immunol 17: 1439-45.

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