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Novel saliva biomarkers for stress and infection in pigs: Changes in oxytocin and procalcitonin in pigs with tail-biting lesions

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ABSTRACT

There is a need for feasible and reliable measures to improve and evaluate production animal health and welfare. Oxytocin is a promising novel stress-related biomarker and procalcitonin may be a measure of sepsis. Both have potential for use in pigs and can be measured from saliva, which allows on-farm sampling with minimal impact on the animals. The current study sought to further validate these measures using a spontaneous situation that causes both stress and an increased risk for infections in pigs, namely a tail-biting outbreak. Grower pigs on a commercial farm belonging to three different phenotype groups were selected: control pigs from control pens (CC, $N = 30$), control pigs (CTB, $N = 10$), and pigs with tail lesions from pens with a tail-biting outbreak (LTB, $N = 27$). A single sample of saliva was collected from each pig and analysed for a range of biomarkers related to stress, infection, inflammation, and immune activation. Oxytocin tended to be higher in CC pigs than in LTB pigs, while cortisol was higher in CTB than CC pigs. Procalcitonin tended to be higher, and haptoglobin was higher in LTB than in CC pigs. Adenosine-deaminase levels were similar between phenotypes. These results provide further evidence for the link between stress and tail biting, and indicate that tail-biting lesions are potential routes for systemic spread of bacteria. Further research into saliva oxytocin as a stress biomarker and saliva procalcitonin as a sepsis biomarker in pigs is warranted.

1. Introduction

There is a need to develop feasible, reliable, and valid measures of animal health and welfare to improve on-farm diagnostics and to assess the welfare of domestic animals (Guevara et al., 2022). Saliva biomarkers are promising alternatives to more intensive methods, such as blood sampling, as they can be collected with minimal stress to the animal (Cerón et al., 2022).

Cortisol is one of the most commonly used biomarkers for both acute and chronic stress (Merlot et al., 2011; Bahnsen et al., 2021). Cortisol can reliably be analysed from saliva (Escribano et al., 2012). However, not only stressors such as transport (López-Arjona et al., 2020), but also physical exercise can increase cortisol secretion (Allgrove et al., 2008). Further, cortisol secretion can be influenced by the sampling procedure (Ruis et al., 1997), and individual variation and the diurnal secretion pattern of cortisol makes baseline sampling especially challenging (Merlot et al., 2011).

One interesting novel biomarker is oxytocin, a neuropeptide with an important role in reproduction, lactation, and maternal behaviour (Lee et al., 2009). Oxytocin is also linked to social affiliation and has anxiolytic, antinociceptive, and stress-buffering effects (Tops et al., 2007; Tops et al., 2012). Oxytocin is centrally released in response to stress and reduces activity of the hypothalamic-pituitary-adrenal (HPA) axis (Lee et al., 2009). Cortisol and oxytocin are thus interrelated in some contexts; oxytocin increases and provides negative feedback due to increased HPA activation (Quintana and Guastella, 2020; Alley et al., 2019). In domestic animals, oxytocin has primarily been studied during positive experiences, especially social ones (Rault et al., 2017). Recently, López-Arjona et al. (2020, 2021) developed two novel oxytocin assays using a monoclonal and a polyclonal antibody and revealed decreased post-stress oxytocin levels in pigs.

Procalcitonin (PCT) is a widely used biomarker of sepsis in human medicine. PCT is a potential tool for non-invasive detection of sepsis in pigs and has recently been validated for use in pig saliva (López-

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Martínez et al., 2022). PCT levels were increased in both a lipopolysaccharide-induced pig model of sepsis and in pigs with meningitis.

Tail biting in pigs is a problematic abnormal behaviour which can be induced by stressful conditions, particularly in intensive housing systems (Edwards and Valros, 2021). Tail biting and the resulting lesions are connected to increased stress and pain in pigs (Munsterhjelm et al., 2013; Sandercock et al., 2019) and can cause local, secondary, and systemic infections (Boyle et al., 2021). Tail-biting lesions are linked to higher levels of acute phase proteins (APPs) (Heinonen et al., 2010; Carroll et al., 2018) and to higher skin temperature in the tail region (Teixeira et al., 2020), indicating that the lesions cause inflammatory responses.

While it is challenging to separate the underlying stress causing tail biting and the stress caused by tail biting per se, it can be assumed that individuals in a pen with an ongoing tail-biting outbreak suffer from a higher total stress level than in pens with no tail biting. Therefore, our aim was to use spontaneously occurring tail biting as a real-life model to assess oxytocin as a biomarker of chronic stress in pigs. In addition, this model was used to test PCT in saliva as a potential measure of sepsis in pigs. These analytes were compared with more traditional biomarkers related to stress, inflammation, and immune activation, namely cortisol, haptoglobin (Hp), and adenosine-deaminase (ADA) and its isoenzymes. The two main hypotheses were that animals in pens with ongoing tail biting would have lower baseline oxytocin levels but higher cortisol levels than animals from control pens, and that pigs with tail lesions are prone to infections and have increased PCT, Hp, and ADA levels.

2. Material and methods

The study protocol was considered ethically acceptable by the University of Helsinki Viikki Campus Research Ethics Committee (Statement 2/2022).

2.1. Animals and housing

The study was conducted in the growing unit of a commercial piglet-producing farm in Southwest Finland. All pigs were born in similar conditions in standard farrowing crates with partly slatted flooring. Pigs were moved to the growing unit at an age of approximately 27 days. Male pigs were castrated, and pigs were not docked.

Pigs were housed in two-climate pens in groups of about 27 pigs per pen. Feeding was ad libitum with liquid feed from sensor troughs (trough length 2.5 m per pen). Water was available from one cup drinker per pen. Pens were 11.4 m^2 in total, of which 7.6 m^2 was solid concrete floor and the remainder slatted. There was a roofed resting area of 3 m^2 at the back end of the pens. A thin layer of peat bedding covered part of the solid floor area. Peat and a small amount of hay or straw was added to the pens one to two times a day, but hay or straw was barely visible in the pens during sampling. Further, each pen contained a hanging chain with four plastic chewing objects attached to it.

Tail health was assessed at the pen level; pens with >10% of pigs in the pen with a fresh tail lesion >0.5 cm were counted. This situation was true for 7.5 pens per section (median; min = 3, max = 15 of 16 pens per section). At least one shortened, healed tail was present in 0.5 pens per section (median; min = 0, max = 3).

2.2. Selection criteria

Animals were housed in a total of six different rooms. From each room, one to three pens with signs of fresh tail biting were chosen (TB) along with a similar number of control pens with no or very mild signs of tail lesions (C). However, in all rooms it was not possible to find enough C pens to match exactly the number of TB pens. In total, 10C pens from five rooms and 13 TB pens from six rooms were included. From each C pen, three pigs were convenience sampled; these were the first pigs to approach the sampler voluntarily or to allow the sampler to approach without withdrawing. Pigs in the TB pens were additionally separated into three different phenotype groups (Table 1). Whenever suitable pigs could be identified, one, or in some cases two pigs were sampled per phenotype per pen. Only pigs that otherwise appeared clinically healthy were included.

Table 1
Selection criteria and pigs included in the study.

Phenotype	Definition	Number of pigs (F:M ratio)[a]	Number of pens pigs originated from	Number of rooms pigs originated from
Control in control pen (CC)	Pig with clinically intact tail from control pens with no or very mild tail lesions.	30 (18:12)	10	5
Control in tail-biting pen (CTB)	Pig with clinically intact tail[b] from pens including pigs with fresh tail lesions.	10 (9:1)	10	5
Pig with mild tail lesion (MTB)	Pig from pens including pigs with fresh tail lesions. Tail is (close to) full length with a fresh wound (redness or blood) or a brownish scab approximately <2 cm in diameter.	13 (8:4)	12	5
Pig with severe tail lesion (STB)	Pig from pens including pigs with fresh tail lesions. Tail has a fresh wound (redness or blood) or a brownish scab. The lesion is approximately >2 cm in diameter or the tail is clearly shortened.	15 (3:11)	13	6

[a] Female to male pig ratio. All male pigs were castrated. The sex was unrecorded for two pigs.

[b] Five of the CTB had very minor signs of lesions on the tail, such as a small, barely visible scab, or a slightly scarred, but fully healed tail tip. These pigs were included as no completely intact tails were available in the pen.

2.3. Sampling procedure

The pigs were between 48 and 62 days of age at the time of sampling. Pigs were sampled between 10.00 and 13.00 by two researchers with experience of saliva sampling in pigs. After identifying suitable pens from the corridor, the sampler entered the pen calmly and when necessary slowly walked through the pen to identify suitable pigs without startling the pigs. If necessary, the sampler squatted down to allow pigs to approach and allow sampling. Straw was used to attract the attention of the pigs if necessary. To avoid any effect of sampling on the biomarkers, only pigs that chewed on the sampling sponge voluntarily were included (i.e. either approached the sampler themselves or allowed to sampler to approach without withdrawing). No animals were restrained for sampling.

Sampling was performed by slightly modifying the methods described in López-Arjona et al. (2020). Pieces of polypropylene sponges were held with metal forceps and gently inserted into the pig's mouth, unless the pig started to chew on its own initiative. The pigs were allowed to chew on the sponge for about 30 s. Once the sponge was clearly wet, it was placed in a Salivette tube (Sarstedt, Germany). Tubes were kept cool and centrifuged at 3000 xg at 4 °C for 10 min. Saliva samples were stored at −80 °C until analysis. Samples were sent for

Table 2
Assay methods used for the different biomarkers.

Biomarker	Assay used	Reference
Oxytocin	Direct competitive AlphaLisa with a monoclonal antibody (oxy-mono)	López-Arjona et al. (2020)
	Indirect competitive AlphaLisa with a polyclonal antibody (oxy-poly)	López-Arjona et al. (2021)
Procalcitonin	Indirect competitive AlphaLisa (polyclonal antibody)	López-Martínez et al. (2022)
Cortisol	Indirect competitive AlphaLisa (monoclonal antibody)	López-Arjona et al. (2020)
Haptoglobin	Direct competitive AlphaLisa (monoclonal antibody)	Contreras-Aguilar et al. (2021)
ADA and its isoenzymes ADA1 and ADA2	Spectrophotometric automated assay (Adenosine Deaminase assay kit, Diazyme Laboratories)	Tecles et al. (2018) Contreras-Aguilar et al. (2020)

analysis in a cold box layered with dry ice to the Interdisciplinary Laboratory of Clinical Analysis (Interlab-UMU) at the University of Murcia (Murcia, Spain).

2.4. Biomarker assays

All biomarker assays have been previously described (Table 2).

2.5. Data handling and statistical analysis

All statistical analyses were performed with IBM SPSS v. 28. Biomarkers were checked for normality using Wilk-Shapiro tests and visual estimation. Oxy-mono was found to be non-normally distributed and was thus Log10-transformed to achieve normality.

The difference in biomarkers for pigs with mild (MTB) and severe tail biting lesions (STB) were initially analysed with *t*-tests. These two phenotypes did not differ in any biomarkers and thus were combined for further analysis into a single group of lesioned pigs (LTB).

As it was not possible to find pure control pens in all rooms, some of the control pens also included some pigs with mild lesions (3 pens), healed tail lesions (2 pens), or ear lesions (1 pen). To ensure these pens were still usable as control pens, the biomarkers of pigs from these pens were compared to pigs in the pure control pens with *t*-tests. No differences were observed and thus all control pens were included in subsequent analyses.

As the aim was to compare biomarkers in pigs with tail-biting-related stress, we performed two planned comparisons. First, we compared CC pigs to CTB pigs to assess a possible effect of the tail-biting pen environment. Second, we compared CC pigs to LTB pigs to assess the possible effect of being a victim of tail biting in addition to that of the tail-biting pen environment. Comparisons were performed using separate linear mixed models for each of the biomarkers. Preliminary analyses with *t*-tests showed that there was a sex difference in biomarkers only for oxytocin and cortisol. The models for oxy-mono, oxy-poly, and cortisol thus included phenotype (either CC vs CTB or CC vs LTB) and sex as fixed factors and initially the interaction between sex and phenotype. The interaction was not significant and was thus removed from all final models. Models for all other biomarkers only included phenotype as fixed factor. All models included pen as a random factor. Model residuals were checked for normality. As the model residuals for total ADA and ADA1 when comparing CC pigs with CTB pigs did not show a normal distribution when using original values, these biomarkers were further Log10-transformed and models were rerun with transformed values.

Descriptive statistics for oxy-mono are presented as medians and quartiles from raw data. Other descriptive results are presented as marginal means and standard errors based on model estimations.

To check for correlations between different biomarkers, Pearson's correlations were run separately for all three phenotypes. Log10-transformed data were used for non-normally distributed variables.

P-values <0.05 were considered significant and *P*-values <0.1 as tendencies.

3. Results

It was not possible to identify all phenotypes in all TB pens, and in a few cases more than two LTB pigs were sampled from the same pen. In addition, the sample sizes (Table 3) varied slightly as the amount of saliva was insufficient for analysis all biomarkers. See Table 3 for descriptive data for biomarkers.

The only biomarker that differed between the CC and the CTB pigs was cortisol ($F_{1,37} = 14$, $P < 0.001$) (Fig. 1). Sex was not significant and was thus removed from the final model.

Oxy-poly ($F_{1,20} = 4.0$, $P = 0.06$) and oxy-mono ($F_{1,24} = 4.9$, $P = 0.06$) tended to be higher in CC than in LTB pigs (Fig. 2a and b). For both types of oxytocin, males had higher levels than females (estimated marginal mean 729 (standard error 80) vs 444 (73) ng/mL, $F_{1,59} = 7.8$, $P = 0.007$ and median 3341 (interquartile range 4596) vs 2652 (2291) pg/mL, $F_{1,49} = 4.9$, $P = 0.03$, respectively). PCT concentration tended to be higher in LTB than CC pigs ($F_{1,50} = 3.3$, $P = 0.07$) (Fig. 3a). Cortisol levels did not differ by phenotype ($P > 0.1$) but was higher in male pigs than in female pigs (66 (9.0) vs 46 (8.9) ng/mL, $F_{1,49} = 5.9$, $P = 0.02$). Hp levels were significantly higher in LTB pigs than in CC pigs ($F_{1,33} = 16$, $P < 0.001$) (Fig. 3b). There were no differences between phenotypes in any of the ADA measures (all $P > 0.1$).

Correlations between all biomarkers for the different phenotypes (CC, CTG, and LTB) separately (Table 4a-4c) were mainly positive.

4. Discussion

This study provides additional support for the validity of saliva oxytocin as a measure of stress in pigs. In comparison to the transport

Table 3
Descriptive statistics (mean and standard deviation (SD)) for the measured saliva biomarkers in control pigs in control pens (CC, $n = 30$), control pigs in tail biting pens (CTB, $n = 10$), and lesioned pigs in tail biting pens (LTB, $n = 28$).

	CC			CTB			LTB		
	N	Mean/*median*	SD/*IR*	N	Mean/*median*	SD/*IR*	N	Mean/*median*	SD/*IR*
Oxytocin monoclonal (pg/mL)[a]	28	*3684.2*	*4766.0*	9	*3234.8*	*4263.0*	27	*2644.8*	*2467.0*
Oxytocin polyclonal (ng/mL)	28	647.2	440.1	9	554.2	503.6	27	498.6	319.5
Procalcitonin (ng/mL)	28	3844.6	2847.8	8	3513.3	2416.8	26	5486.7	4019.7
Cortisol (ng/mL)	30	53.2	39.4	10	62.5	60.3	28	47.6	31.1
Haptoglobin (ng/mL)	28	2328.3	1456.7	9	3597.1	1924.9	27	4056.9	1734.2
ADA[b] (U/L)	30	1240.3	1012.7	10	1388.7	717.3	28	1676.2	991.8
ADA[b]1 (U/L)	30	1222.8	1011.2	10	1369.6	718.0	28	1659.8	990.1
ADA[b]2 (U/L)	30	17.6	10.9	10	19.1	11.7	28	16.3	8.2

[a] Monoclonal oxytocin values did not fit a normal distribution and thus median and interquartile range (IR) are given.
[b] Adenosine-deaminase.

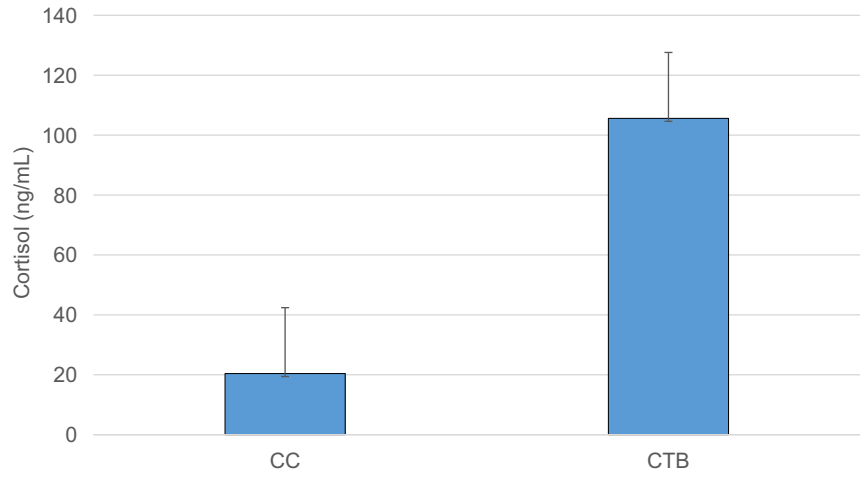

Fig. 1. Estimated marginal means and standard error for cortisol (ng/mL) in control pigs from control pens (CC) and control pigs from tail-biting pens (CTB). The phenotypes differ significantly ($P < 0.001$).

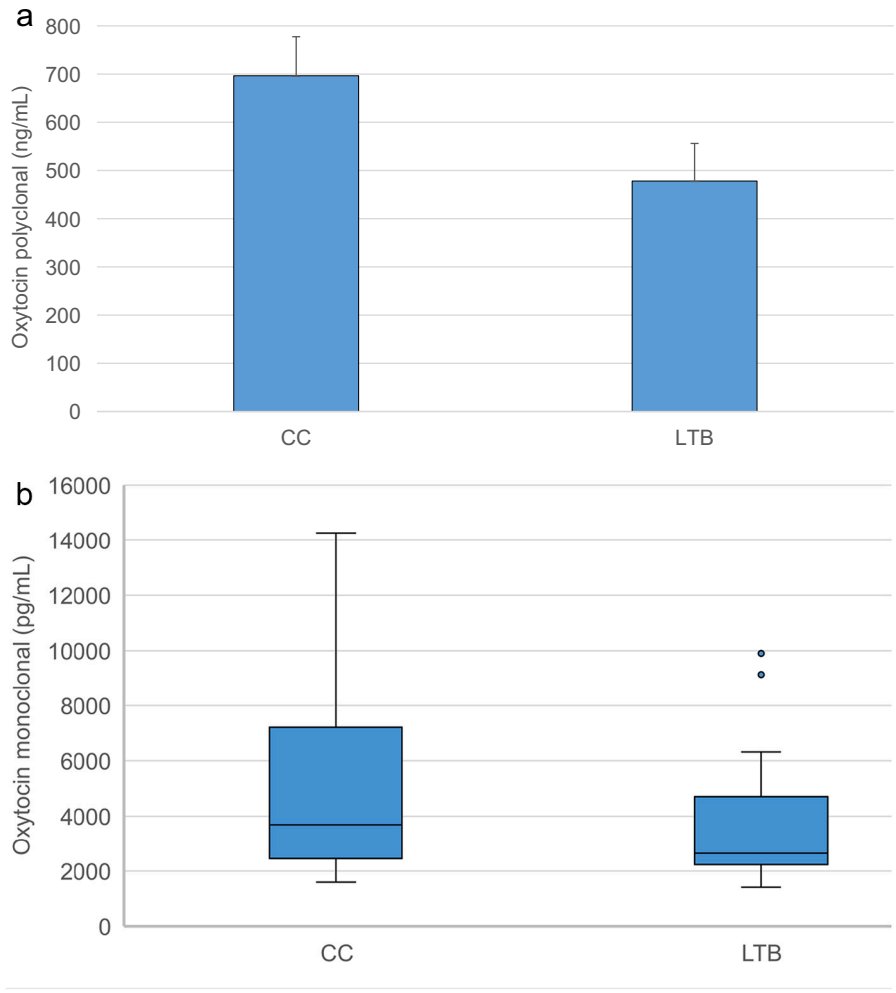

Fig. 2. (a) Estimated marginal means and standard error for polyclonal oxytocin (ng/mL) and (b) boxplot based on original values for monoclonal oxytocin (pg/mL) in control pigs from control pens (CC) and lesioned pigs from tail-biting pens (LTB). The two phenotypes tend to differ for both oxytocin measures ($P = 0.06$ for both).

stress modelled by López-Arjona et al. (2020), this study compared baseline, home pen samples of pigs of different phenotypes. Oxytocin tended to be lower in pigs with tail-biting lesions compared to control pigs in control pens, indicating that these pigs may suffer from more stress, or, alternatively, less positive states.

We found a tendency for lower oxytocin concentration in LTB than CC pigs, while there was no difference in cortisol. Oxytocin and cortisol can be interrelated, with oxytocin suggested as a buffer of stress reactions (Tops et al., 2007; Tops et al., 2012). Oxytocin secretion is related to social situations and affects social bonding (Lee et al., 2009;

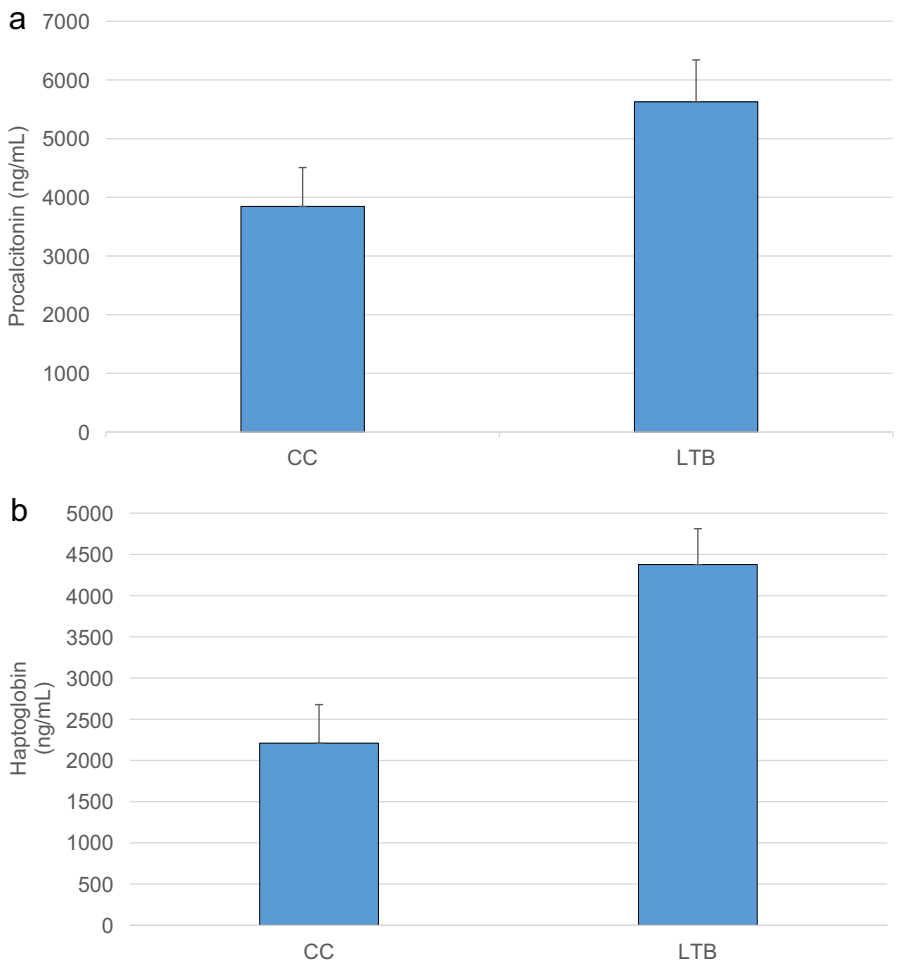

Fig. 3. (a) Estimated marginal means and standard error for procalcitonin (ng/mL) and (b) haptoglobin (ng/mL) in control pigs from control pens (CC) and lesioned pigs from tail-biting pens (LTB). The phenotypes tended to differ for procalcitonin ($P = 0.07$) and differed significantly in haptoglobin levels ($P < 0.001$).

Table 4a
Correlations between biomarkers including controls in control pens (CC) (n = 30). Significant correlations ($P < 0.05$) are indicated by bolding and tendencies ($P < 0.1$) by bolded italics.

		Oxytocin polyclonal (pg/mL)	Procalcitonin (ng/mL)	Cortisol (ng/mL)	Haptoglobin (ng/mL)	ADA[a] (U/L)	ADA[a]1 (U/L)	ADA[a]2 (U/L)
Oxytocin monoclonal (pg/mL)	r_p	**0.86**	**0.61**	**0.50**	**0.56**	**0.39**	***0.38***	**0.47**
	P	**<0.001**	**<0.001**	**0.007**	**0.002**	**0.04**	***0.05***	**0.01**
	n	**28**	**26**	**28**	**28**	**28**	***28***	**28**
Oxytocin polyclonal (ng/mL)	r_p		**0.46**	**0.62**	**0.64**	0.28	0.27	**0.58**
	P		**0.02**	**<0.001**	**<0.001**	0.15	0.17	**0.001**
	n		**26**	**28**	**28**	28	28	**28**
Procalcitonin (ng/mL)	r_p			−0.05	0.26	0.02	0.02	0.26
	P			0.82	0.21	0.92	0.93	0.19
	n			28	26	28	28	28
Cortisol (ng/mL)	r_p				0.34	0.26	0.25	**0.48**
	P				0.08	0.17	0.18	**0.007**
	n				28	30	30	**30**
Haptoglobin (ng/mL)	r_p					0.15	0.14	**0.52**
	P					0.46	0.48	**0.005**
	n					28	28	**28**
ADA[a] (U/L)	r_p						**1.0**	0.15
	P						**<0.001**	0.43
	n						**30**	30
ADA[a]1 (U/L)	r_p							0.14
	P							0.47
	n							30

[a] Adenosine-deaminase.

Table 4b
Correlations between biomarkers including controls in tail biting pens (CTB) (n = 10). Significant correlations ($P < 0.05$) are indicated by bolding.

		Oxytocin polyclonal (pg/mL)	Procalcitonin (ng/mL)	Cortisol (ng/mL)	Haptoglobin (ng/mL)	ADA[a] (U/L)	ADA[a]1 (U/L)	ADA[a]2 (U/L)
Oxytocin monoclonal (pg/mL)	r_p	**0.99**	0.38	**0.93**	0.43	−0.08	−0.82	**0.73**
	P	**<0.001**	0.41	**<0.001**	0.25	0.85	0.83	**0.03**
	n	**9**	7	**9**	9	9	9	**9**
Oxytocin polyclonal (ng/mL)	r_p		0.41	**0.95**	0.50	−0.14	−0.15	**0.76**
	P		0.37	**<0.001**	0.2	0.72	0.71	**0.02**
	n		7	**9**	9	9	9	**9**
Procalcitonin (ng/mL)	r_p			0.6	−0.82	0.31	0.24	0.34
	P			0.12	0.86	0.94	0.96	0.41
	n			8	7	8	8	8
Cortisol (ng/mL)	r_p				0.42	0.1	0.09	**0.81**
	P				0.27	0.79	0.81	**0.004**
	n				9	10	10	**10**
Haptoglobin (ng/mL)	r_p					−0.16	−0.16	0.54
	P					0.69	0.68	0.14
	n					9	9	9
ADA[a] (U/L)	r_p						**1.0**	−0.06
	P						**<0.001**	0.88
	n						**10**	10
ADA[a]1 (U/L)	r_p							−0.07
	P							0.84
	n							10

[a] Adenosine-deaminase.

Table 4c
Correlations between biomarkers including pigs with tail lesions (LTB) (n = 28). Significant correlations ($P < 0.05$) are indicated by bolding and tendencies ($P < 0.1$) by bolded italics.

		Oxytocin polyclonal (pg/mL)	Procalcitonin (ng/mL)	Cortisol (ng/mL)	Haptoglobin (ng/mL)	ADA[a] (U/L)	ADA[a]1 (U/L)	ADA[a]2 (U/L)
Oxytocin monoclonal (pg/mL)	r_p	**0.82**	−0.14	**0.43**	**0.56**	0.13	0.12	**0.63**
	P	**<0.001**	0.52	**0.02**	**0.003**	0.52	0.54	**<0.001**
	n	**27**	25	**27**	**27**	27	27	**27**
Oxytocin polyclonal (ng/mL)	r_p		−0.06	**0.57**	**0.52**	0.33	0.32	**0.73**
	P		0.76	**0.002**	**0.005**	0.10	0.10	**<0.001**
	n		25	**27**	**27**	27	27	**27**
Procalcitonin (ng/mL)	r_p			***−0.39***	0.09	0.06	0.06	−0.06
	P			***0.05***	0.69	0.76	0.76	0.79
	n			***26***	25	26	26	26
Cortisol (ng/mL)	r_p				0.25	0.11	0.11	**0.70**
	P				0.22	0.58	0.59	**0.01**
	n				27	27	27	**27**
Haptoglobin (ng/mL)	r_p					0.11	0.11	**0.40**
	P					0.58	0.59	**0.04**
	n					27	27	**27**
ADA[a] (U/L)	r_p						**1.0**	0.21
	P						**<0.001**	0.29
	n						**28**	28
ADA[a]1 (U/L)	r_p							0.20
	P							0.31
	n							28

[a] Adenosine-deaminase.

Lürzel et al., 2020). Thus, the results suggest that LTB pigs were under more social stress. Alternatively, these results may reflect a decrease of positive states in pigs suffering from tail biting. In addition to the underlying stress behind the outbreak, tail biting causes the victim pigs stress and pain (Munsterhjelm et al., 2013; Sandercock et al., 2019). Oxytocin is often referred to as the 'feel-good' hormone or as an indicator of positive emotions (Mitsui et al., 2011). In addition, oxytocin has analgesic effects (Xin et al., 2017), with lower oxytocin concentrations in humans in pain (Oladosu et al., 2020). However, oxytocin regulation and its effects remain poorly understood (Rault et al., 2017) and further studies are warranted.

CTB pigs did not have different oxytocin levels than CC pigs, although the values were numerically intermediate to the two other phenotypes. However, cortisol was higher in CTB than CC pigs. The results from CTB pigs should be interpreted with caution. First, half of the pigs were actually not pure controls due to practical constraints but had very minor signs of lesions upon close inspection of the tail. Three CTB pigs had a barely visible scab on their tail tip, and two had signs of a very mild previous tail injury, with healed scar tissue at the tail tip. Secondly, as the CTB pig was sometimes the only pig in the pen with an intact tail, it is possible that these pigs were actually biters. We did not systematically try to identify biters. Munsterhjelm et al. (2013) revealed that biters may have an increased stress level, which may explain this result. On the other hand, if the CTB pigs were true 'neutrals' (i.e. neither biters nor victims), one additional explanation may be that pigs change their behaviour to stay neutral during a tail biting outbreak, such as by reducing feed intake (Palander et al., 2013), which may be stressful. Finally, cortisol may not be very informative due to its diurnal variation (Merlot et al., 2011). However, in our study the animals were sampled in a similar diurnal secretion phase.

Consistent with several previous studies (Brown et al., 2016), we observed a correlation between cortisol and oxytocin. However, López-

Arjona et al. (2020) did not find a correlation between cortisol and oxytocin in their transport study. This is consistent with the results of Alley et al. (2019), who observed a correlation only in baseline but not in post stress-treatment values. The very high correlation in especially CTB pigs ($r > 0.9$), which also had higher cortisol levels than CC pigs, suggests differing regulation of the feedback from oxytocin on the HPA axis. A possible dysregulation, seen as correspondingly high or low levels of both oxytocin and cortisol, was shown in connection to post-traumatic stress disorder in humans (Li et al., 2019).

The increased levels of PCT in LTB pigs suggests a potential spread of bacteria from the tail lesions to the bloodstream (Sihvo et al., 2012). In humans, although PCT can identify severely infected ulcers (Jeandrot et al., 2008), PCT can also increase in certain traumas per se (Parli et al., 2018). Therefore, further studies are needed to establish PCT ranges for confirming infection in pigs. In addition, further research should be performed to elucidate the reason of the strong correlation between oxytocin and procalcitonin that appeared in CC pigs only.

The higher Hp in LTB than in CC pigs is not surprising, given that similar results were observed in previous studies (Heinonen et al., 2010; Carroll et al., 2018; Petersen et al., 2002) and Hp is a validated marker of inflammation, trauma, and infection (Cerón et al., 2022). In addition, Hp has been suggested as a biomarker for stress (Salamano et al., 2008). The latter may also explain why Hp levels were numerically higher in CTB when compared with CC pigs and the positive correlation with oxytocin. In addition, there is increasing evidence of ill-health being a risk factor for tail biting (Nordgreen et al., 2020). It cannot be excluded that the pigs in the TB pens were suffering for subclinical illness. Respiratory diseases and lameness are possible health-related risk factors for tail biting (Boyle et al., 2021) and both are linked to an increased level of Hp (Petersen et al., 2002; Gutiérrez et al., 2009).

Although ADA and its isoenzymes did not show significant variation between groups, ADA2 correlated with Hp and also with oxytocin and cortisol especially in pigs from TB pens. This could indicate a relation between ADA and stress, as previously reported in pigs where ADA1 and ADA2 correlated with pain score in lame and prolapsed pigs (Contreras-Aguilar et al., 2019). This link warrants further investigation.

Due to practical constraints, only pigs that voluntarily chewed on the sampling sponge were included. These pigs are potentially bold pigs with a high motivation for exploration. Previous studies have shown that tail-biting behaviour is linked to an explorative phenotype (Ursinus et al., 2014; Haigh et al., 2020). Thus, it is possible that the sampling procedure influenced the results. However, Haigh et al. (2020) did not find a difference in baseline cortisol levels between bold and shy pigs. Further, the procedure was the same for all phenotypes included in the study, which should minimize the risk of bias.

This was a first pilot study to explore the relationship between tail-biting phenotype and oxytocin and procalcitonin and the number of animals used was limited by practicalities. However, due to the high variability of these biomarkers, a larger number of animals should be included in future studies to corroborate these pilot findings. Based on post hoc power analyses, the sample size to achieve significant results for oxytocin and procalcitonin would be approximately 50–70 animals per phenotype. Furthermore, we did not perform blood cultures to verify if the higher concentrations of PCT in LTB pigs was linked to sepsis. Future studies should test the possibility of using biomarkers related to stress as predictors of tail-biting outbreaks, and for identifying pigs in which the lesion caused a systemic bacterial infection. In addition, repeated individual measurements of the biomarkers would be recommended especially for monitoring purposes and for better understanding the co-regulation of the different biomarkers.

5. Conclusions

The results of this study indicate that oxytocin and procalcitonin may be potential biomarkers of stress and sepsis in tail biting in pigs. In addition, these results provide further support for the link between stress and tail biting and previous data on tail-biting lesions being potential routes for systemic spread of bacteria.

Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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