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# The use of social network analysis to describe the effect of immune activation on group dynamics in pigs

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ABSTRACT

The immune system can influence social motivation with potentially dire consequences for group-housed production animals, such as pigs. The aim of this study was to test the effect of a controlled immune activation in group-housed pigs, through an injection with lipopolysaccharide (**LPS**) and an intervention with ketoprofen on centrality parameters at the individual level. In addition, we wanted to test the effect of time relative to the injection on general network parameters in order to get a better understanding of changes in social network structures at the group level. 52 female pigs (11–12 weeks) were allocated to four treatments, comprising two injections: ketoprofen-LPS (**KL**), ketoprofen-saline (**KS**), saline-LPS (**SL**) and saline-saline (**SS**). Social behaviour with a focus on damaging behaviour was observed continuously in 10 × 15 min bouts between 0800 am and 1700 pm 1 day before (baseline) and two subsequent days after injection. Activity was scan-sampled every 5 min for 6 h after the last injection in the pen. Saliva samples were taken for cortisol analysis at baseline and at 4, 24, 48, 72 h after the injections. A controlled immune activation affected centrality parameters for ear manipulation networks at the individual level. Lipopolysaccharide-injected pigs had a lower *in-degree centrality*, thus, received less interactions, 2 days after the challenge. Treatment effects on tail manipulation and fighting networks were not observed at the individual level. For networks of manipulation of other body parts, *in-degree centrality* was positively correlated with cortisol response at 4 h and lying behaviour in the first 6 h after the challenge in LPS-injected pigs. Thus, the stronger the pigs reacted to the LPS, the more interactions they received in the subsequent days. The time in relation to injection affected general network parameters for ear manipulation and fighting networks at the group level. For ear manipulation networks, *in-degree centralisation* was higher on the days following injection, thus, certain individuals in the pen received more interactions than the rest of the group compared to baseline. For fighting networks, *betweenness* decreased on the first day after injection compared to baseline, indicating that network connectivity increased after the challenge. Networks of tail manipulation and manipulation of other body parts did not change on the days after injection at the group level. Social network analysis is a method that can potentially provide important insights into the effects of sickness on social behaviour in group-housed pigs.


## Implications

Damaging behaviour is a major welfare problem in pig husbandry and has been linked to poor health. We were able to detect changes in social interactions in response to a controlled immune activation both on individual and group levels using social network analysis.

## Introduction

When animals become sick, pro-inflammatory cytokines can alter social motivation so that they can withdraw, conserve resources and recover (reviewed by Nordgreen et al., 2020). In

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intensive pig production systems, animals housed in close confinement cannot withdraw from their pen mates when they experience a bout of illness, and this might influence their social interactions (Veit et al., 2021). There are indications that poor health status is positively correlated with damaging behaviours (Moinard et al., 2003; Taylor et al., 2012). In particular, it was shown that pigs diagnosed with respiratory diseases tended to perform more ear- and tail biting than controls in the days prior to disease outbreak (Munsterhjelm et al., 2017). These so-called damaging behaviours are supposed to spread either actively due to social learning (Blackshaw, 1981) or passively through animals encountering a wounded tail or ear (Fraser, 1987). Damaging behaviours have an unpredictable appearance and rapid spread, as well as a sporadic occurrence which makes them difficult to study (reviewed by D'Eath et al., 2014). So far, most studies of damaging behaviours focus either on pen-level data (Larsen et al., 2019; Li et al., 2020) or dyadic interactions (Brunberg et al., 2011; Zonderland et al., 2011; Munsterhjelm et al., 2016), or related indicators, such as tail posture (Zonderland et al., 2009; Lahrmann et al., 2018). Except for studies on the relationship between indirect genetic effects for growth rate and biting behaviour (Camerlink et al., 2015), studies that take the whole group of animals into account are lacking.

Social network analysis (**SNA**) provides standardised mathematical methods for calculating measures of sociality across levels of social organisation and has become an increasingly common tool for studying animal behaviour (reviewed by Makagon et al., 2012). Social network analysis is widely used in different fields (e.g. primatology, behavioural ecology, epidemiology) and across many species but most extensively in wildlife research (Stanton and Mann, 2012; Aplin et al., 2013; Brent et al., 2013) and to a much lesser extent in captive farm animals (Abeyesinghe et al., 2013; Boyland et al., 2016). Previous studies in pigs have focused on agonistic behaviour such as the description of general network properties (Büttner et al., 2015a) and individual network position (Büttner et al., 2015b) across three mixing events. Social network analysis is relevant for animal welfare and farm management, Foister et al. (2018) were able to predict long-term aggression (3 week postmixing) by calculating network properties at 24 h after mixing. Studies on damaging behaviours such as ear- and tail biting (Li et al., 2018) are underrepresented even though SNA has the potential to shed light on underlying social mechanisms and the spread of these behaviours. Social network analysis variables of particular interest in this context are *degree centrality* and *edge density*. *Degree centrality* is measured at individual level and is determined by how many interactions this pig has with others. *Edge density* is measured at group level and indicates how well the members of the group are connected in terms of their interaction with each other (Foister, 2019). After a controlled immune activation, pigs exhibited a shift in social motivation and performed more ear and tail manipulation 2 days after the challenge (Munsterhjelm et al., 2019). Based on these findings, we would expect a higher *out-degree centrality* in ear and tail manipulation networks of challenged pigs as well as a higher *edge density* in the networks on the days following a controlled immune activation.

Lipopolysaccharide (**LPS**) is a part of the cell wall of Gram-negative bacteria (e.g. *Escherichia coli*) and can be used to model aspects of sickness. Lipopolysaccharide binds to toll-like receptors (TLRs) on several types of immune competent cells and activates the innate immune system within an hour after administration. As a first response, interleukin-1, interleukin-6, tumour necrosis factor $\alpha$, interleukin-8, C-reactive protein and cortisol are released. The pro-inflammatory cytokines give rise to sickness behaviour and an increase in prostaglandin synthesis through the enzyme cyclooxygenase as well as a profound reduction in activity and increase in cortisol during the first 6 h after injection (Nordgreen et al., 2018; Veit et al., 2021). In rodents, depressive-like behaviour after overt sickness has been observed (O'Connor et al., 2009). In pigs, more ear and tail manipulation and changes in central cytokine and monoamine levels have been reported within 2 to 3 days after LPS-injection (Nordgreen et al., 2018; Munsterhjelm et al., 2019; Veit et al., 2021). Non-steroidal anti-inflammatory drugs such as ketoprofen are able to lower the effect of LPS on cortisol release and attenuate behavioural signs of sickness (Veit et al., 2021). Non-selective non-steroidal anti-inflammatory drugs inhibit cyclooxygenase-1 and -2 thereby prostaglandin $E_2$ synthesis (Thompson et al., 2018).

Due to relatively small group sizes (1–3 pigs), previous studies (Nordgreen et al., 2018; Munsterhjelm et al., 2019) were unable to fully mimic the housing conditions on farms, where pigs are kept in larger groups (six and more), thus, the complexity of social interactions that could be studied was limited. In this study, we therefore wanted to further understand how pig social behaviour is influenced when one member of a larger group becomes ill and thereby changes its behaviour. To achieve this, we used social network analysis to test the effect of a controlled immune activation and an intervention with ketoprofen on centrality parameters (e.g. *degree centrality*) on pig level. In addition, we wanted to test the effect of time relative to injection on general network parameters (e.g. *edge density*) in order to get a better understanding of changes in social network structures on pen level. We hypothesised that an injection with LPS affects the standing of an individual pig in a group of pen mates and that illness in one pig changes the group dynamics after recovery. We predicted that the centrality parameters in ear and tail manipulation networks of a challenged pig would be affected in a way that the number of interactions received (*in-degree*) decreases, whereas the number of interactions initiated (*out-degree*) increases (I). Moreover, we predicted that the number of interactions within a group (*edge density*) would increase the subsequent days after challenge (II). We applied this method to continuous observations of social behaviour that were gathered during a previous experiment (Veit et al., 2021).

## Material and methods

### *Animals and husbandry*

The experiment took place between March 23th and May 15th 2018 at the Livestock Production Research Center of the Norwegian University of Life Sciences (NMBU), campus Ås. 78 undocked pigs aged between 11 and 12 weeks were used in two blocks (52 females and 26 castrated males). Animal caretakers selected four females and two males per litter as even in size as possible to be group-housed in the 13 pens they were born in at a stocking density of 1.3 $m^2$ per pig. The four female pigs in each pen were randomly allocated to one of four treatments each, so that all treatments were represented in all pens, resulting in 13 pigs per treatment. The male pigs were companion pigs used to increase the stocking density and group size. Housing details are provided in Veit et al. (2021).

### *Experimental design*

The description of the design is adapted from Veit et al. (2021). The pigs were allocated to four treatments comprising to injections: ketoprofen-LPS (**KL**), ketoprofen-saline (**KS**), saline-LPS (**SL**), saline-saline (**SS**), Fig. 1. Ketoprofen (6 mg/kg) or saline (similar volume of 0.9%) was injected into the *trapezius* muscle. Lipopolysaccharide (1.2 μg/kg, serotype 0111: B4 of *E. coli* dissolved in 0.9% sterile saline to a concentration of 100 μg/ml,

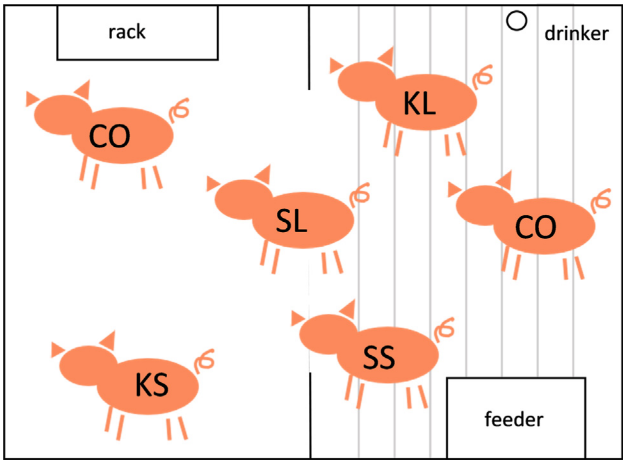


**Fig. 1.** Pen design (shaded area = slatted flooring) and treatments of pigs (KL = ketoprofen-lipopolysaccharide, KS = ketoprofen-saline, SL = saline-lipopolysaccharide, SS = saline-saline and CO = companion).

produced by Sigma, Germany) or a similar volume of saline was administered intravenously through an ear vein catheter on average 60 ± 14 min afterwards. Saliva samples were taken by letting the pigs chew on a cotton pad at baseline and at 4, 24, 48, 72 h after the intravenous injection. Details about sampling procedures are described in Veit et al. (2021). One camera per pen (door ccd-camera, Smartprodukter, Ulsteinvik, Norway) was placed centrally on the ceiling above and the pigs were individually marked on the back. The Media Recorder system from Noldus (Wageningen, the Netherlands) was used to run video recordings of behaviour continuously throughout the experiment.

*Cortisol analysis*

Cortisol concentration in saliva has been measured in a previous study (Veit et al., 2021). In brief, an enzyme immunoassay kit according to the manufacturer's protocol was used (DetectX®, Catalogue Number K0033-H5W, Arbor Assays, MI, USA). The optical density of each well was read with the Sunrise Absorbance Reader (Tecan Austria GmbH, Grödig/Salzburg, Austria) at 450 nm using the Magellan 6.4 software. Mean coefficient of variation varied between 4.69 and 7.63%. Sensitivity was determined as 27.6 pg/ml and limit of detection was determined as 45.4 pg/ml according to manufacturer.

*Video analysis*

All behavioural video recordings have been analysed in a previous study (Veit et al., 2021) using the Observer XT 14.1 from Noldus (Wageningen, The Netherlands). The methods applied are described here for completeness. Behavioural signs of sickness were observed by instantaneous scan sampling (Altmann, 1974) every 5 min for 6 h after the injection of the last pig in the pen (**DAY1**). The observer was blinded to treatment. The frequency of lying lateral/ sternal/ alert and being active was included as a measure for the response strength to LPS. Social behaviour was observed at baseline (1 day before injection), referred to as **DAY0**, and on the first and second day after injection (**DAY2** and **DAY3**). Continuous observations of performers and receivers of social behaviour at certain intervals during the day were performed by one observer who was blinded to treatment and day of experiment. The sampling scheme for DAY0, DAY2 and DAY3 was four 15 min intervals in the morning between 8:00 and 10:00 and six 15 min intervals in the afternoon between 14:00 and 17:00, (Fig. 2). The day of injection itself (DAY1) was not of interest for observation of social behaviour because it was interrupted due to more handling on that day. Only 12 out of 13 pens were included in analysis due to an inadequate quality of the video material from one pen (which was too brightly lit to identify back markings). The ethogram for the specific social behaviours performed is displayed in Table 1. Due to low frequencies, the behaviours flank nosing (4.9% of all behaviour observed) and belly nosing (4.1%) as well as displacement (2.1%) were not used for further analyses.

The package *igraph* in R 4.0.3 was used to construct networks and calculate network properties at pig and at pen level (Table 2). Centrality parameters were obtained via the *degree* and *eigen_centrality* functions and normalised by the pig with the highest value in the respective pen, thus, centrality was scaled between 0 and 1 (1 = most central pig in the pen). General network parameters were obtained via the *edge_density, centralization.degree, centralization.betweenness* and *centralization.evcent* functions. *Degree centralisation* was normalised by the most central pig in the respective pen. Codes for calculation of network parameters are given in the Supplementary Material S1.

*Statistical analysis*

JMP Pro 14.3.0 (SAS, NC, USA) was used to build mixed models for analysis of network parameters. The significance level for all analyses was set at $P < 0.05$. Residuals were checked for normality and homogeneity of variance by visual inspection of plots. Main effects are not presented when the interaction was in focus to answer the research question. *A priori* planned contrasts were used after running the main models, as we had predefined assumptions (for further explanation see Doncaster and Davey, 2007). For centrality parameters, the calculated values of *degree centrality, in-degree centrality, out-degree centrality* and *eigenvector centrality* were used as dependent variables. The treatment (KL, KS, SL, SS), the day (DAY0, DAY2, DAY3) and the interaction of both were used

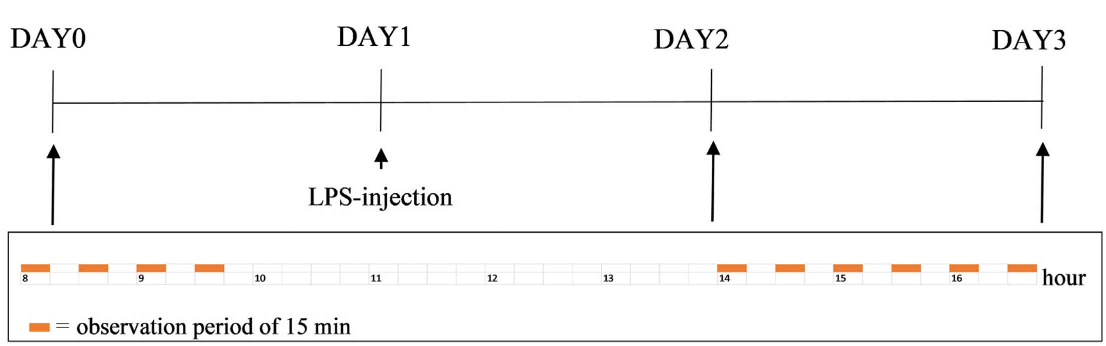


**Fig. 2.** Schematic overview of the sampling scheme for observation of pig social behaviour over the 3-day experimental period (LPS = lipopolysaccharide).

**Table 1**
Ethogram for social behaviour in pigs.

| Behaviour | Description |
| --- | --- |
| Ear manipulation | Touching the ear of another pig with the snout, including taking the ear into the mouth |
| Tail manipulation | Touching the tail of another pig with the snout, including taking the tail into the mouth |
| Manipulation of other body parts | Touching body parts of another pig with the snout except for tail, ear, belly and flank region (e.g. head, legs, back), including taking the body parts into the mouth |
| Fighting | Biting, hitting, and knocking of another pig with the head. Includes chasing performed immediately after biting, hitting, knocking. Includes parallel pressing after knock, hit or bite. Pig that initiates the fight is the performer, pig that is being attacked is the recipient |
| Flank nosing | Touching the flank region (=upper part of the lateral side of the body from the beginning of the shoulder until the end of the body, except of tail) of another pig with the snout |
| Belly nosing | Repetitive up and down movements on the abdomen of another pig that is lying or standing |
| Displacement | Pushing away another pig without fighting (as defined above) results in active movement of the recipient and getting access to a resource (e.g. silage, lying space, drinker) for the performer |

as independent fixed effects. Pig nested in treatment was included as a random variable in all models. Companion pigs were not considered for analysis. For planned comparisons, Student's *t*-tests were used. In a first step, we compared SL with SS to elucidate the effect of LPS on centrality parameters. In addition, the comparison of SL and KL should answer the question whether ketoprofen alleviates the effects of LPS. Furthermore, it was relevant to compare SS with KS in order to see whether ketoprofen has an effect in pigs that were not challenged with LPS. If any of these pairwise comparisons were significant, we compared within-group differences between baseline and the day at which the significant treatment effect was found (Veit et al., 2021). For correlations between centrality parameters and cortisol concentrations at 4 h after injection, as well as general activity in the first 6 h after injection, Spearman rank coefficient was used. For general network parameters, the calculated values of *edge density, degree centralisation, in-degree centralisation, out-degree centralisation, betweenness* and *eigenvector* were used as dependent variables. The day (DAY0, DAY2, DAY3) was used as independent fixed effect, and the pen was included as a random variable in all models. For planned comparisons, Student's *t*-tests were used. Codes for statistical models are given in the Supplementary Material S2.

## Results

### General description of the data set

Lipopolysaccharide activated the hypothalamic–pituitary-adrenal axis as indicated by an increase in salivary cortisol at 4 h after injection and depressed activity within 6 h; ketoprofen alleviated this effect (reported in Veit et al., 2021). Pigs across all treatments and days manipulated mostly the ears (23.7% of all behaviours observed) and other body parts (31.8%) of their pen mates and were frequently involved in fights (24.4%). Tail manipulation was shown to a much lesser extend (9.0%). An overview of the different centrality parameters at pig level calculated by treatment and day is displayed in the Supplementary Table S1 and the results are described in detail in the following paragraph. An overview of the general network parameters calculated at pen level for all behaviours by day is displayed in the Supplementary Table S2 and the results are described subsequently.

### Effect of treatment on centrality parameters (pig level)

The calculated centrality parameters were used to plot social networks for each pen and day (Fig. 3). Lipopolysaccharide had a significant effect on centrality parameters of ear manipulation at an individual level ($F(\text{treatment*day})_{6,\,87.53} = 1.82$, $P = 0.11$). SL pigs (median (min|max) = 0.40 (0.10|1.00)) had a significantly lower *in-degree centrality*, thus, received less interactions, compared to SS pigs (0.83 (0.40|1.00)) 2 days after injection (planned comparison: $P = 0.01$), Fig. 4a. A pretreatment with ketoprofen did not alleviate this effect. A numerical difference between SL and SS pigs was present at baseline. Neither LPS nor ketoprofen had an effect on centrality parameters of tail manipulation (Fig. 4b), manipulation of other body parts (Fig. 4c) and fighting (Fig. 4d) and no clear patterns could be observed. *In-degree centrality* of SL pigs for manipulation of other body parts was positively and significantly correlated with salivary cortisol concentration at 4 h after injection (DAY2: Spearman's rho $\rho = 0.71$, $P = 0.009$) and lying laterally in the first 6 h after the challenge (DAY3: $\rho = 0.62$, $P = 0.03$). Thus, the stronger the pigs reacted to the LPS, the more interactions they received in the subsequent days.

**Table 2**
Social network parameters based on observations of social behaviour in pigs.

| Terminology | Description |
| --- | --- |
| Centrality parameters (pig level) | |
| Degree centrality | Number of direct interactions an individual has with other individuals of the group |
| In-degree | Number of interactions received by an individual |
| Out-degree | Number of interactions initiated by an individual |
| Eigenvector centrality | Takes the degree centrality of an individual, as well as the degree centrality of other individuals it is connected with, into account |
| General network parameters (pen level) | |
| Edge density | Amount of actual interactions between individuals divided by the total number of possible interactions in the group. An edge represents the interaction between two individuals. |
| Degree centralisation | The range or variability of the individuals' centrality values (0 indicates that all individuals in the network have equal centrality; 1 indicates maximum inequality) |
| In-degree | Description of whether certain individuals receive more interactions than the rest of the group |
| Out-degree | Description of whether certain individuals initiate more interactions than the rest of the group |
| Betweenness | Pens with high values contain individuals who connect other individuals that do not directly interact |
| Eigenvector | Pens with high values contain a small number of well-connected individuals, with the rest of the group being considerably less well-connected |

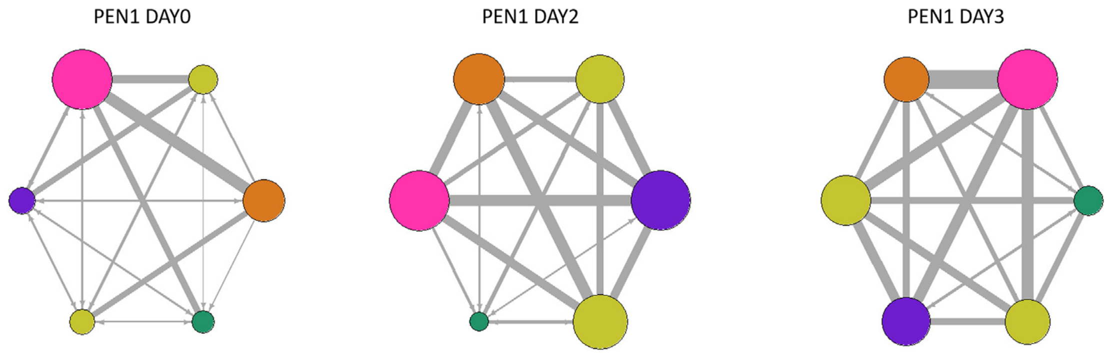


**Fig. 3.** Example of a social network based on all interactions of pigs observed in pen 1 at baseline (DAY0) and on the first (DAY2) and second day (DAY3) after injecting the pigs with ketoprofen-lipopolysaccharide (KL), ketoprofen-saline (KS), saline-lipopolysaccharide (SL) and saline-saline (SS). Nodes represent individuals in the pen, colour of the nodes indicate treatments (pink = SL, blue = KS, orange = KL, green = SS, yellow = companion) and size of the nodes represents *degree centrality*; edges represent interactions between individuals, arrows point from the actor to the receiver and thickness of the edges represents the frequency. In this example, the KS pig increases its *degree centrality* from baseline to DAY2 and DAY3 (but to a lesser extent than at DAY2), whereas the SL pig largely has unchanged *degree centrality*. The frequency of interactions within the network increases from baseline to DAY2 and DAY3. The pattern differed from pen to pen, and this illustration is meant as an aid in understanding.

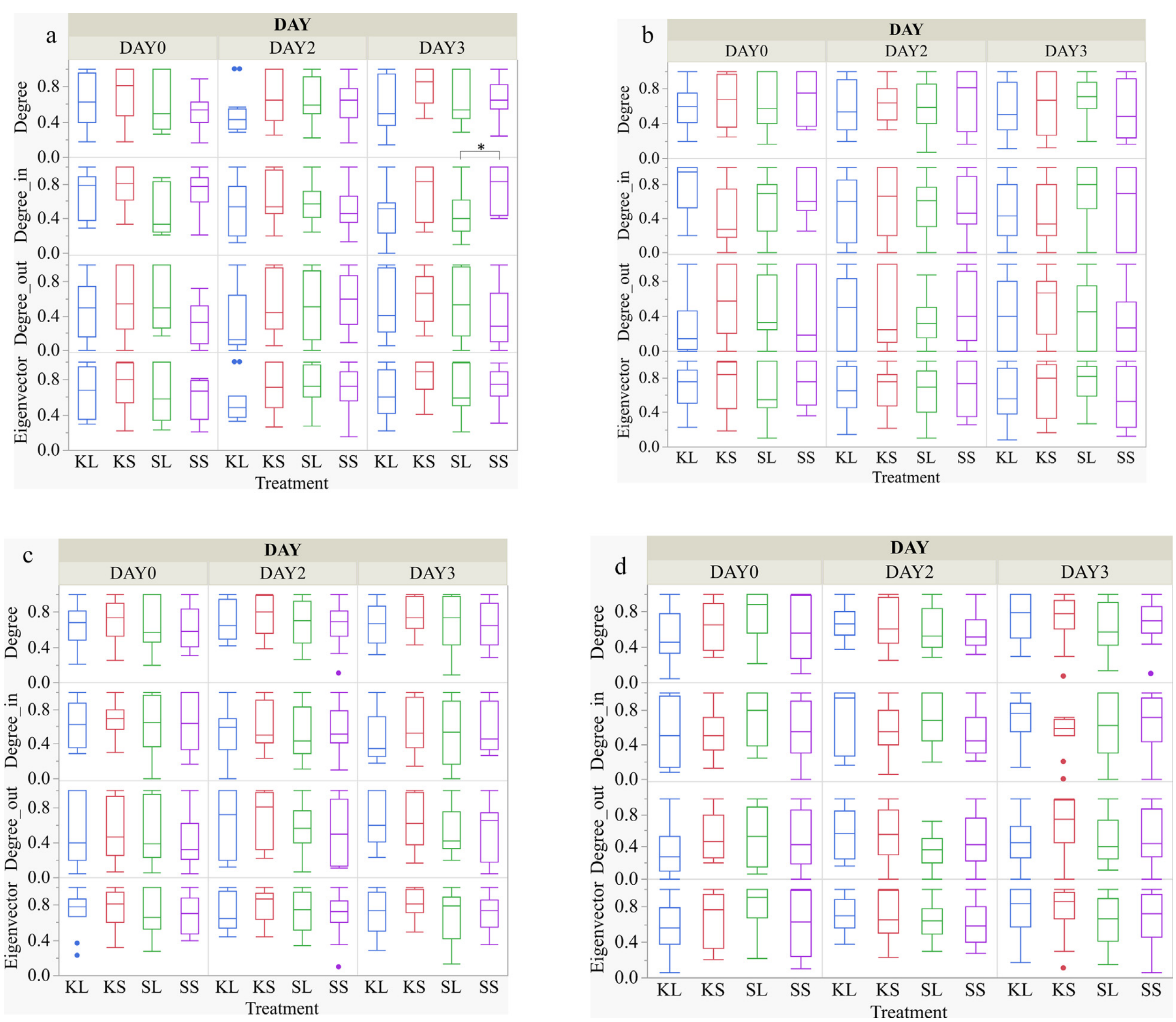


**Fig. 4.** Centrality parameters calculated for ear manipulation (a), tail manipulation (b), manipulation of other body parts (c) and fighting (d) at baseline (DAY0) and at the first (DAY2) and second day (DAY3) after injecting pigs with ketoprofen-lipopolysaccharide (KL), ketoprofen-saline (KS), saline-lipopolysaccharide (SL) and saline-saline (SS). Round dots represent outliers. Significant differences of planned comparisons between treatments ($P < 0.05$) and within day are marked with an asterisk (*).

*Effect of time relative to injection on general network parameters (pen level)*

Day relative to injection had an effect on general network parameters of ear manipulation. On the first (median (min|max) = 0.41 (0.26|0.57)) and second day after injection (0.45 (0.28|0.67)), *in-degree centralisation* ($F(day)_{2,22}$ = 4.74, $P$ = 0.02) was significantly higher compared to baseline (0.33 (0.10|0.54), planned comparisons DAY0 vs DAY2: $P$ = 0.04; DAY0 vs DAY3: $P$ = 0.007), Fig. 5a. Thus, certain individuals in the pen received more interactions than the rest of the group. Day relative to injection had an effect on general network parameters of fighting. 1 day after injection, *betweenness* ($F(day)_{2,22}$ = 2.85, $P$ = 0.08) was significantly lower (0.15 (0.04|0.44)) compared to baseline (0.26 (0.07|0.48), planned comparison: $P$ = 0.03), Fig. 5b, indicating that network connectivity increased following the injection. Day relative to injection had no effect on general network parameters of tail manipulation and manipulation of other body parts (Fig. 5).

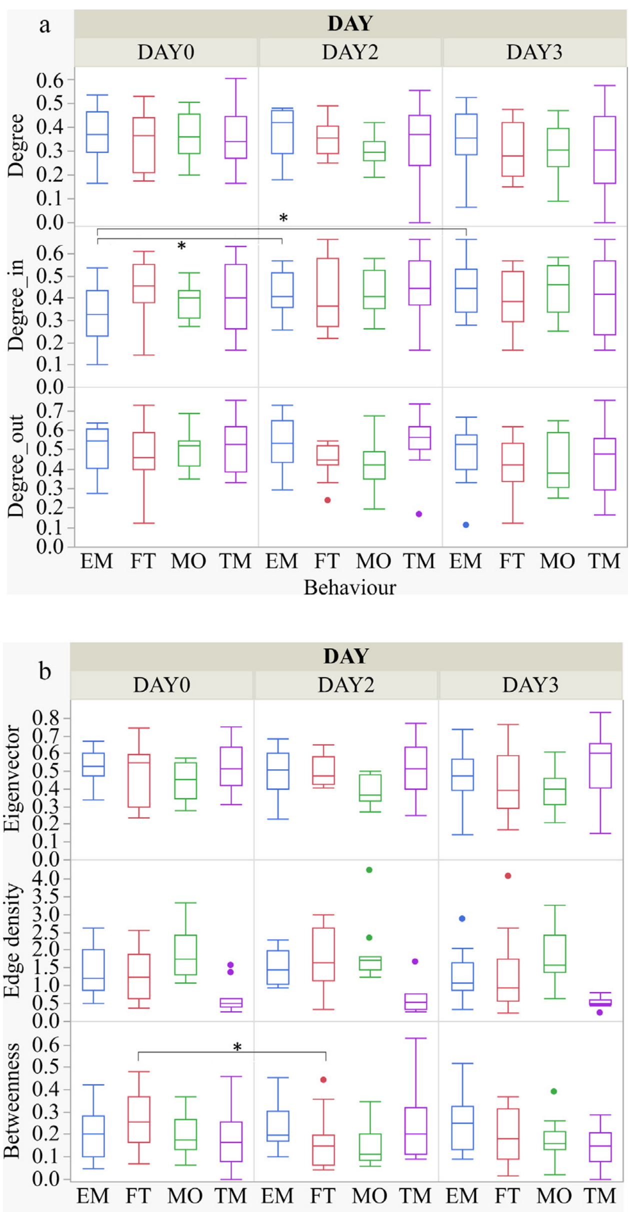


**Fig. 5.** General network parameters (a, b) calculated for ear manipulation (EM = blue boxplot), fighting (FT = red), manipulation of other body parts (MO = green) and tail manipulation (TM = lilac) over 3 days in pigs. Round dots represent outliers. Significant differences of planned comparisons between days ($P$ < 0.05) are marked with an asterisk (*).

## Discussion

### Summary

We were able to detect changes in social interactions in response to a controlled immune activation at both individual (pig) and group (pen) levels using social network analysis. For ear manipulation networks, an injection with LPS resulted in a lower *in-degree centrality* 2 days after the challenge at pig level, meaning that the ears of LPS-injected pigs were manipulated to a lesser extent compared to saline-injected pigs. Treatment effects on tail manipulation and fighting networks were not observed. Ketoprofen seemed not to have an impact on centrality parameters at pig level. For networks of manipulation of other body parts, *in-degree centrality* was positively correlated with cortisol response and lying behaviour in the first 6 h after the challenge in LPS-injected pigs. This finding indicates that the stronger the pigs reacted to the challenge, the more manipulations were directed towards them on the following days. At the pen level, a higher *in-degree centralisation* for ear manipulation networks in the two subsequent days after injection compared to baseline was found, thus, certain individuals were more frequently manipulated than the rest of the group. For fighting networks, *betweenness* decreased on the first day after injection compared to baseline, indicating that network connectivity increased following the injection. Time relative to injection had no effect on general network parameters of tail manipulation and manipulation of other body parts.

### General aspects

The proportions of different social behaviours observed in this study were similar to other studies in group-housed pigs. Bolhuis et al. (2006) found that manipulative oral behaviour directed at pen mates in 15- and 19-week-old pigs mainly consisted of manipulating other body parts (58% of total observations on manipulative behaviour) and ear biting (30%) whereas belly nosing (8%) and tail biting (4%) were observed less frequently (fighting was not included). Also, Van der Meer et al. (2017) showed that oral manipulation in 20- and 23-week-old pigs was directed mainly towards other body parts and ears, while to a lesser extent towards tails or belly. Slightly contradictory, Camerlink and Turner (2013) observed that nosing between 8-week-old pigs consisted mainly of nose-to-nose contact, nosing the body and nosing the head while nosing the ears was rather uncommon, as was nosing the tails.

Pigs used in the present study were similar in age, and housed litter-wise in the same environment from birth. In commercial pig production, regrouping and rehousing are very common management procedures. The stable housing and social conditions in the present study might have had a general impact on network parameters. It has been shown that piglets in socialised pens showed a significantly lower *degree centrality*, *eigenvector centrality* and *clustering coefficient* compared to controls (Turner et al., 2020). We applied SNA to a rather small group of individuals (six) compared to the group sizes of previous studies (8 in Li et al., 2018, 6–29 in Büttner et al., 2015b, 15 in Foister et al., 2018, 12 in Turner et al., 2020), which limits the number of possible interactions within the group. Nevertheless, this is the first study that takes a variety of behavioural patterns into account when describing the effects of immune activation.

*Effect of treatment on centrality parameters (pig level)*

It was shown in rodents that when sickness behaviour resolves, mice display depressive-like behaviours measured by increased immobility in the forced swim test and tail suspension test at 24–28 h after LPS-challenge (Frenois et al., 2007; O'Connor et al., 2009; Ge et al., 2015; Zhu et al., 2015; Sulakhiya et al., 2016; Zhao et al., 2019). It is these psychological aftereffects and their potential effect on social interactions that we wanted to investigate with the current experiment in pigs. Immune activation has been suggested as a major factor influencing social interactions in pigs, with outbreaks of damaging behaviours such as tail biting as a possible result (reviewed by Nordgreen et al., 2020). The shift in social motivation (seen as more tail and ear directed behaviour) was observed about 40 h after the signs of acute illness dissipated and was not accompanied by a similar increase in activity (Munsterhjelm et al., 2019). In boars, tail- and ear biting tended to increase 0–2 weeks before clinical signs of respiratory infection were visible (Munsterhjelm et al., 2017), thus, behaviour changed already in a preclinical stage of illness. This could also be the case in the phase of recovery when clinical signs abate. Thus, pigs might feel irritable, which might increase the probability to become a biter. Irritability, emotional lability and short temper are reported side effects in humans undergoing cytokine therapy (Denicoff et al., 1987; Renault et al., 1987; Capuron et al., 2000; Constant et al., 2005).

In a previous study with the same pigs, we found that LPS-injected pigs manipulated the ears of their pen mates longer compared to saline-injected pigs on the second day after injection (Veit et al., 2021). In the present study, LPS-injected pigs received fewer ear manipulations 2 days after the challenge compared to saline-injected pigs. The previous results are based on the duration of the behaviour, whereas the SNA is based on the frequencies of the respective behaviour. It appears logical, that pigs that perform longer ear manipulations are less likely a target for ear manipulations themselves. Thus, SNA provides a different perspective on the behavioural effect of LPS. It has been discussed that a 'predamage' state (Fraser and Broom, 1997), in which pigs perform so-called 'tail-/ear-in-mouth behaviour' (Schrøder-Petersen et al., 2003; Diana et al., 2019), can develop into a 'damage-state'. Thus, the gentle tail or ear manipulation we observed could be a precursor of more severe biting behaviour. Camerlink and Turner (2013) found that nosing the tail correlated with tail biting and nosing an ear correlated with ear biting. Nevertheless, severe tail or ear lesions were not observed in the present study.

Lipopolysaccharide activates the hypothalamic–pituitary-adrenal axis as indicated by a peak in cortisol concentrations at 4 h after injection (Webel et al., 1997; Nordgreen et al., 2018). In the present study, the cortisol response and behavioural signs of sickness on the day of injection were correlated with centrality parameters calculated on the first and second day after the challenge. We found that the stronger the cortisol response at 4 h and the more frequently pigs were lying on their side in the first 6 h after the injection, the more these pigs were manipulated by their pen mates on the following days when they were recovered from the challenge. Exposure to stressors is commonly associated with increased hypothalamic–pituitary-adrenal axis activity, and therefore, the response of cortisol is generally considered an indicator of stress (Dallman et al., 1987; Sapolsky et al., 2000). Stress has been found to significantly affect the physiology and behaviour of captive and wild populations, which can alter individual behaviour and overall network structure (Boogert et al., 2014). When it comes to interpreting manipulation of other body parts, Jensen and Wood-Gush (1984) suggested a threatening function of 'nose-to-nose' contact and associated 'nose-to-body' contact with individual recognition. Camerlink and Turner (2013) found that nosing other parts of the body was unrelated to damaging forms of interaction. It is therefore not clear whether manipulation of other body parts can be interpreted as purely affiliative social behaviour.

Ketoprofen reduces prostaglandin $E_2$ production in LPS-injected pigs and inhibits thereby a fever response (Wyns et al., 2015). This is one possible pathway through which ketoprofen can influence behaviour. Moreover, some non-steroidal anti-inflammatory drugs are able to alter the expression of NFkappaB and thereby reduce subsequent cytokine expression (Peters et al., 2012), but whether ketoprofen works in this way is not known. Ketoprofen alleviated the effect of LPS on sickness behaviour on the day of injection but did not affect social network parameters in the subsequent days after the challenge. However, the pigs that were injected with LPS and ketoprofen did not change their behaviour in the same way that the pigs that received LPS without ketoprofen did.

*Effect of time on general network parameters (pen level)*

At pen level, high *degree centralisation* describes whether certain individuals initiate or receive more interactions than the rest of the group. High *betweenness centralisation* occurs where subgroups within a pen interact only indirectly through a small number of intermediary animals (Turner et al., 2020). In the present study, *in-degree centralisation* has increased the days following injection for ear manipulation networks, thus, the ears of certain pigs were manipulated more often than their pen mates' compared to baseline. *Betweenness* was decreased for fighting networks 1 day after injection, suggesting that interactions were more evenly spread across all group members and no single individual was responsible for connecting a fragmented network. *Edge density* increased only numerically for ear manipulation and fighting networks on the day after injection compared to baseline. *Edge density* indicates how well the members of the group are connected in terms of their interaction with each other and we expected it to increase in all networks the days after injection.

Behavioural changes can be seen in sick animals but also in their healthy social companions. Lipopolysaccharide-injected pigs and pigs diagnosed with osteochondrosis received increased social attention by pen mates (Munsterhjelm et al., 2017 and 2019). On the other hand, LPS-injected pigs performed more tail and ear directed behaviour than their controls in the subsequent days after the challenge (Munsterhjelm et al., 2019). The increased interest in sick animals during the first hours seems logical, but the mechanisms behind the observed change in social behaviour in the following days need to be investigated in future research.

In other studies using social network analysis, the effect of mixing (Büttner et al., 2015a and 2015b), feed-restriction (Cañon Jones et al., 2010) and higher stocking density (Cañon Jones et al., 2011) was tested at group level. In the present study, all treatments were represented in each pen, which hampers the interpretation of general network parameters. A change in group dynamics in the days following injection could be interpreted as a result of a behavioural change of one pig in the group (SL) or as a reaction to the handling of the group as a whole. Even so, the study provides insights on the effects of sickness on social behaviour, where there is still a paucity of scientific literature.

## Conclusion

There might be long-lasting effects on social behaviour, both at individual and group levels, when even just one individual in a group becomes ill. Changes were detected in ear manipulation and fighting, which together cover 48% of observed social behaviours, thus, a significant part of social activity was affected. The results indicate that the pigs changed the way they directed social activities, and that the immune status of individuals affected these changes. This needs to be considered in studies of effects of health on behaviour when animals are kept in groups and shows a need for further studies on how individuals in a group should be managed when they become ill.

## Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.animal.2021.100332.

## Ethics approval

The animal study was reviewed and approved by the NMBU IACUC and the food safety authorities (FOTS ID 15232).

## Data and model availability statement

None of the data were deposited in an official repository. Data are made available upon request.

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## Declaration of interest

None.

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