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Fractal measures in activity patterns: Do gastrointestinal parasites affect the complexity of sheep behaviour?



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ABSTRACT

Gastrointestinal nematodes are known to be one of the most economically important parasites in livestock production. In order to test whether fractal analysis of behaviour can be used as a diagnostic tool for detection of infected animals, we investigated fractal patterns in the behavioural activity of domestic sheep (*Ovis aries*) in relation to strongylid infection. Temporal dynamics in activity patterns of 20 sheep were recorded at high resolutions using tri-axial accelerometer loggers attached to the neck of naturally infected subjects. We measured fractal dynamics in the resultant acceleration time series, divided into periods of activity and inactivity, using several fractal methods and tested the prediction that temporal complexity in the activity patterns of infected control sheep and experimentally dewormed sheep should differ. Detrended fluctuation analysis (DFA) revealed that sheep behaviour sequences were characterized by long-range correlations, meaning that acceleration fluctuations are not random but depend on long-term activity events. Generalized linear mixed models built to test for the effect of deworming on fractal estimates showed that the temporal organization of sheep activity varies with the status of strongylid infection. Our results indicate that sheep treated with anthelmintics exhibited a higher complexity in their activity sequences than parasitized sheep, suggesting that organizational patterns of their behaviour change with gastrointestinal parasite infection. Thus, we provide evidence for the potential utility of fractal methods in behavioural welfare monitoring.

1. Introduction

Gastrointestinal nematode infections have considerable welfare and economic costs in livestock production worldwide (Charlier et al., 2014a,b), principally through sub-clinical morbidity. In small ruminants, nematode infections have been reported to cause diarrhoea, weight loss, reduced growth rate, abortion, infertility, lower wool production and milk yield, while heavy infection can lead to the death of the animal (Parkins and Holmes, 1989; Louie, 2007; Mavrot et al., 2015). The typical control of these infections by frequent use of anthelmintic treatments has led to an alarming rise of drug-resistance (Papadopoulos et al., 2012; Rose et al., 2015; Sharma and Ganguly, 2016). Multiple drug resistance has been recorded repeatedly all over

the world for the three most important genera of nematodes infecting small ruminants: *Teladorsagia*, *Haemonchus* and *Trichostrongylus* (Jackson et al., 2009; Papadopoulos et al., 2012). This major threat to production, along with recent changes in the distribution and abundance of livestock parasitic nematodes attributed to climate change (Kenyon et al., 2009; Dijk et al., 2010; Fox et al., 2012, 2015), calls for rapid improvement of parasite monitoring programs and control strategies. For example, targeted, selective treatment strategies, where animals that require anthelmintics are identified and then treated accordingly, have a key role to play in maintaining drug efficacy (Gaba et al., 2010; van Wyk et al., 2006; Charlier et al., 2014a,b). The development of early diagnostic systems that would detect infected individuals from the asymptomatic phase would greatly contribute to the

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implementation of such disease control measures.

Measuring behavioural changes is probably one of the most valuable ways to achieve early disease detection (Dawkins, 2004; Weary et al., 2009). Being non-invasive and non-disruptive, behavioural monitoring has gained increasing interest for assessing the health status of farmed animals and should enable the development of methodologies that will objectively detect potential welfare issues (Nasirahmadi et al., 2017). For example, some recent work has demonstrated that monitoring chicken flock behaviour with cameras could provide an early warning-system for infection with pathogenic intestinal bacteria (Colles et al., 2015). Moreover, using video image analysis and sensors, Martínez-Avilés et al. (2017) could detect altered movements of pigs experimentally infected with African swine fever virus, sometimes before any observation of clinical signs was possible.

Innovative sensor technologies offer high precision and resolution for data sampling, which, together with the application of modern statistical methods, offers the potential to objectively quantify subtle and sometimes otherwise undetectable changes in behavioural patterns. This opens a promising avenue toward the development of automated behavioural health monitoring (Matthews et al., 2016; Neethirajan, 2017). Moreover, for the last two decades, some advanced analytical tools have been discussed as promising techniques to detect stress or illness when no clear clinical signs can be observed (Rutherford et al., 2004; Asher et al., 2009). Among these recent methodologies, fractal analysis has been repeatedly demonstrated to be an indicator of health condition in a wide variety of situations and species. Interestingly, it has been shown to detect subclinical conditions that could not have been revealed using conventional statistics on standard behavioural measures (Hocking et al., 2007; Kembro et al., 2009b; Rutherford et al., 2003; Cribb and Seuront, 2016; Burgunder et al., 2017).

The application of this analytical tool, derived from complexity theory, relies on the fundamental fractal properties (i.e. scale invariance) that characterise animal behaviour in both its temporal and spatial organization. Fractal structures or processes exhibit patterns that repeat themselves across a range of different measurement scales. These scale-invariant patterns can be observed in the timing of specific behaviours (Shimada et al., 1995; Cole, 1995; Alados and Weber, 1999; MacIntosh et al., 2011), as well as in animal movement paths (Bartumeus 2007; Sims et al., 2008; Viswanathan et al., 2011). Health advantages and behavioural flexibility have both been associated with fractal-like patterns, because they are thought to enable organisms to better cope with unpredictable internal or external perturbations (MacIntosh, 2014, 2015; West, 1990). For example, fractality in the bronchial architecture of mammals is more tolerant to fluctuations in the physiological environment during morphogenesis (West, 1990). Furthermore, under novel environmental conditions, both fruit flies, *Drosophila melanogaster* (Shimada et al., 1995), and Japanese quail, *Coturnix coturnix* (Kembro et al., 2009a), produce fractal patterns in sequences of foraging behaviour to better explore and exploit resources. Complexity in a system's structural organization is defined by its fractal scaling, that is to say, by the degree to which fluctuations in a system's behaviour change with the scale of measurement. Numerous studies have shown a loss of complexity (i.e. less irregularity) in biological systems under stressed or pathological conditions. Indeed, reduced variability in space or over time through ageing and disease has been demonstrated in many human physiological, cognitive and motor systems (Lipsitz and Goldberger, 1992; Ribeiro et al., 2002; Vaillancourt and Newell, 2002; Benoit et al., 2011). Among domestic animals, lower complexity (i.e. greater periodicity or stereotypy) in temporal dynamics has been reported in the heart rate of sheep experiencing pain (Stubsjøen et al., 2010) and of swine during hemorrhagic shock (Batchinsky et al., 2007). Concerning animal behaviour, fractal analyses, in particular, have revealed lowered complexity in behaviours of stressed or physiologically challenged individuals, in numerous species (see reviews in Seuront, 2010, 2015; MacIntosh, 2014; Seuront and Cribb, 2017). Related analyses even show reductions in the complexity

of different plant structures, via developmental instability, and spatial arrangements under stressful conditions such as overgrazing and desertification (Eghball et al., 1993; Alados et al., 1999; Escos et al., 1995; Sinclair et al., 2015).

However, despite several promising findings and the potential utility such approaches could have in behavioural monitoring, relationships between physiological or psychological states and altered behavioural complexity remains under-explored, and under-developed. In this study, we investigated fractal patterns in the behavioural activity of domestic sheep (*Ovis aries*) in relation to gastrointestinal parasite infection, with the aim of further testing whether fractal analysis of behaviour can be used as a diagnostic tool for detection of parasitized, and therefore potentially compromised animals. We focused on strongylid nematodes, a group that includes some of the most economically important parasites in production settings, and their influence on temporal dynamics in activity patterns in our subjects. Animal-attached data-loggers equipped with triaxial accelerometers were used to record at high temporal resolutions the activities of naturally infected animals, and relate them to a subset of animals treated with commercial anthelmintics to remove parasite infections. We measured the fractal dynamics of these acceleration time series, divided into periods of activity and inactivity, and tested the prediction that temporal complexity in the activity patterns of naturally infected control sheep and experimentally dewormed sheep should differ, i.e. that parasite infection causes altered fractal complexity in sheep behaviour.

2. Methods

2.1. Data collection

The study was conducted from 11 September to 14 October 2015, in eastern Czech Republic (49°12'06"N, 18°06'42"E). Among a herd of 61 domestic sheep, 20 ewes were randomly chosen and assigned to either a 'control' group (n = 10), or a 'treatment' group (n = 10). The age of the selected sheep ranged from 1 to 8 years. At all times, the animals were free to roam within an enclosed 3 ha pasture.

The experimental period was divided into 3 periods of 7 days each: (i) a pre-treatment control period, at the end of which an anthelmintic treatment (ivermectin, Bimectin® 1%, Vétoquinol, France) was applied to the animals in the treatment group using standard protocols; (ii) a post-treatment transient period during which activity data were not recorded; and, (iii) a post-treatment period. Before the post-treatment period, we verified that treatment was effective by checking the treated sheep for strongylid infection. One treated animal was still found positive for strongylid infection, with an estimated intensity of infection falling within the range of the intensity of infection of untreated sheep. It was therefore assigned to the 'control' group (n = 11) instead of the 'treatment' group (n = 9).

Faecal sampling was carried out by manually immobilizing each ewe and collecting about two grams of fresh stool directly from their rectum to assure identification and avoid possible contamination from other faeces on the ground. The infection status of the 20 subjects was assessed a week before the pre-treatment period, as well as on the first day and last day of each period. The fresh faecal samples were then immediately stored in a small plastic bag and carried in a cooler box for later examination at the Department of Pathology and Parasitology, University of Veterinary and Pharmaceutical Sciences of Brno, Czech Republic.

Temporal sequences of behaviour were recorded using tri-axial (orthogonal) accelerometers (Axy-3; 9 × 15 × 4 mm, 0.7 g, Technosmart s.r.l. www.technosmart.eu) set to record acceleration ($\pm 4g$) at 10 Hz. Neck-mounted devices were fixed with black waterproof Pattex® tape to collars and hung below the head of the sheep.

2.2. Analyses

2.2.1. Parasitological examination

Within the 3 days following their collection, the faecal samples were microscopically examined for the presence of strongylid eggs using a McMaster method described in Kalousova, 2013. Briefly, a weighted amount of faeces was suspended in 2 ml of saturated sodium chloride. After faecal suspension, the supernatant was transferred into a double-chambered McMaster slide and examined at 10x magnification. All strongylid eggs within both chambers were counted and the number of eggs per gram of faeces (EPG) calculated. A sample was recorded as positive if at least one egg was observed using this examination method. Initially, all subjects selected for this study were found to be naturally infected with strongylids (*Nematodirus* spp., *Protostrongylus* spp. and *Muellerius* spp.).

2.2.2. Data analysis

All data processing and statistical analyses were conducted using RStudio1.0.136 (RStudio Team, 2016) in R 3.3.2 (R Development Core Team, 2016).

Vectorial dynamic body acceleration (VeDBA) was used as a measure of activity (Gleiss et al., 2011; Qasem et al., 2012). This activity index was calculated as the vectorial sum of the three dynamic body acceleration (DBA) axes (with x for 'surge', y for 'sway' and z for 'heave'), which were derived by subtracting the static acceleration values from the raw acceleration values (Shepard et al., 2008). Static acceleration values were approximated by applying a box-smoothing function (box size = $20 \cong 2$ s) to the total acceleration recorded for each axis, and VeDBA was then averaged each second.

We performed all the following analyses on acceleration time series that were continuously recorded during the 7-day pre-treatment and post-treatment periods. To avoid potential problems associated with the long periods of inactivity overnight, these full sequences were also divided into shorter, daily time series (06:30–18:30, $N = 13.65 \pm 0.99$ per individual). To analyse temporal dynamics in sheep locomotor activity, we performed 4 different methods of fractal analysis. We used two variants of detrended fluctuation analysis (DFA, Peng et al., 1995), including the linear and the bridge-detrending methods (DFA_b), in addition to the box-counting method (Gneiting et al., 2012) and a modified periodogram method (Taquq et al., 1995). The R package 'fractal' (Constantine and Percival, 2014) was used to perform DFA and the modified periodogram method. The box-counting method was implemented in the 'fractaldim' package (Sevcikova et al., 2014).

In the DFA method, activity time series were first coded as binary time series, with activity denoted by 1 and rest by -1 , at 1-s intervals (Fig. 1). We determined VeDBA threshold values by which to separate activity from inactivity using the interpeak frequency minimum method described in Collins et al. (2015). This method consists of examining the shape of the frequency histograms of acceleration metrics to identify the threshold values which are defined as the values corresponding to the minimum data points falling between peaks (Appendix Fig. 1). These values were determined for each individual and from both the pre-treatment and the post-treatment data. Using accelerometer data, common behaviours that can be discriminated in sheep are high-energy expenditure activities such as grazing, walking and running, and low-energy activities such as standing and lying (Marais et al., 2014; Giovanetti et al., 2017). We defined the sheep's activity here as the time spent active (i.e. grazing, walking and running) and time spent passive (i.e. standing or lying) based on this distinction.

The binary time series were integrated and then divided into non-overlapping windows of equal length n . For the linear-detrending method, a least-squares regression line was fitted to the data in each block to represent their trend. For the bridge-detrending method, a line connecting the first and last data points in each window was drawn to create a slope based only on those data points. The root-mean-square fluctuation, $F(n)$, was then estimated across all windows of varying

sizes. On a double logarithmic plot of $F(n)$ as a function of time scale n (Appendix Fig. 2), the slope of the line determines the scaling exponent α_{DFA} which quantifies long-range dependence (correlation properties) in the time series (Taquq et al., 1995). For $\alpha = 0.5$, the time series is an uncorrelated, random sequence, whereas for $\alpha \neq 0.5$, the sequence shows long-range autocorrelation, meaning that the duration of a behaviour is influenced by the history of the sequence. If $\alpha > 0.5$, the time series is said to be persistent (i.e. positively correlated), with long bouts more likely to be followed by long bouts. If $\alpha < 0.5$, the time series is said to be antipersistent (i.e. negatively correlated), with long bouts more likely to be followed by short bouts and vice-versa. Theoretically, for persistent time series, α_{DFA} is inversely related to the degree of complexity estimated by the fractal dimension: smaller values reflect lower degrees of long-range dependence, or greater stochasticity. For antipersistent time series, smaller α_{DFA} values reflect higher degrees of long-range dependence, or greater determinism. A critical step for interpreting the scaling exponent is to determine the class of long memory process represented in the data sequence (Seuront, 2010). We therefore tested the signal class to determine whether they reflect fractional Gaussian noise (fGn) or fractional Brownian motion (fBm) by examining the scaling exponent, with $\alpha_{\text{DFA}} \in (0,1)$ indicating fGn and $\alpha_{\text{DFA}} \in (1,2)$ indicating fBm.

We determined the appropriate range of time scales within which strong scaling behaviour existed in the sequences using the R^2 -SSR procedure, as described in Seuront, 2010. After validation of the best scaling regions, we applied DFA and DFA_b on these specific regions only.

In the box-counting method, the time series were initially placed in a single box and then divided into smaller and smaller equally sized boxes of size n . At each box size, $N(n)$, the total number of boxes required to cover the curve of the time series entirely, was counted. On a double logarithmic plot of $N(n)$ as a function of n , the slope of the least squares regression line determines the box-counting dimension (FD_b), an estimate of the fractal dimension of the time series (Longley and Batty, 1989; Liebovitch and Toth, 1989). The FD_b is a measure that quantifies the complexity of a signal, i.e. the degree of space filling of a signal in a plane. This dimension lies between 1, when the curve is Euclidean, and 2 when the curve fills the whole space available.

The modified periodogram method estimates the Hurst exponent by a spectral regression analysis (Taquq et al., 1995). Time series were first decomposed into a sum of cosine waves of different frequencies. The frequency axis obtained was divided into blocks of equal size on a logarithmic scale, and the periodogram values corresponding to the frequencies inside each block were then averaged. Finally, the Hurst coefficient H , which is a classic measure of the degree of complexity and long-range dependence of time series (Hurst 1951; Mandelbrot and Van Ness, 1968; Cannon et al., 1997) was estimated by calculating the slope of the line relating the log periodogram values to the log frequencies.

The scaling exponents α_{DFA} and α_{DFA_b} are both theoretically related to the Hurst exponent such that $H \approx \alpha_{\text{DFA}}$ for fGn and $H \approx \alpha_{\text{DFA}_b} - 1$ for fBm. For one-dimensional series like those examined here, the box-counting dimension is related to H such that $H \approx 2 - \text{FD}_b$. However, in practice, the various methods often lead to different H values. For a more robust interpretation of the results, the Hurst exponents were thus calculated separately using the different fractal methods we applied and then averaged (Appendix Table 1), firstly over all the H estimators calculated (mean denoted hereafter as H_1), and secondly over those derived solely from the two DFA methods (mean denoted hereafter as H_2).

Generalized linear mixed models (GLMMs) were built to test the relationship between each scaling exponent (i.e. α_{DFA} , α_{DFA_b} , H_S , FD_b , H_1 and H_2) derived from the daily sequences and those calculated from the full sequences. Models were constructed with Gaussian error structures and logit link functions using the function lmer of the 'lme4' package (Bates et al., 2014). Fractal estimators from the daily sequences

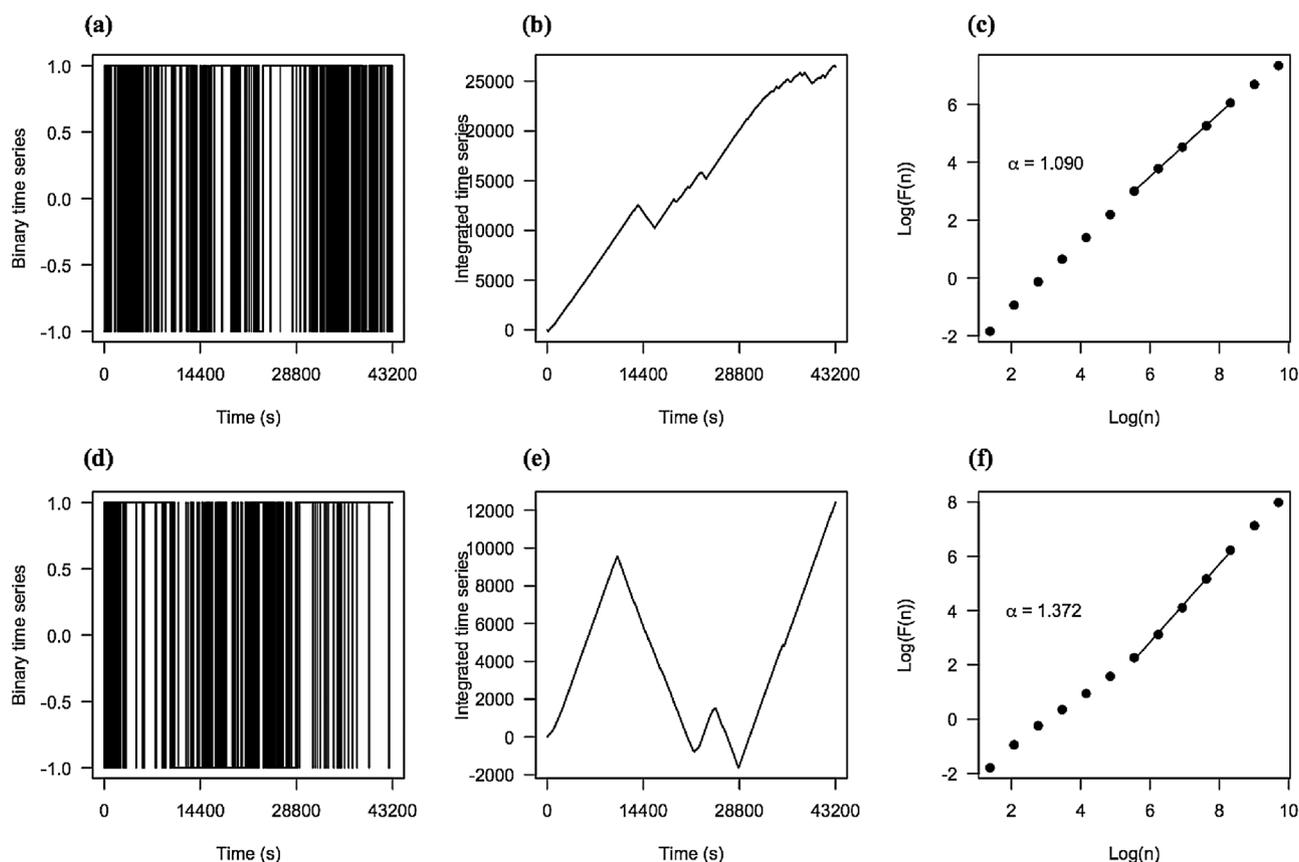


Fig. 1. Detrended fluctuation analysis (DFA) of activity sequences from representative control (infected; first row) and dewormed (second row) domestic sheep following anthelmintic treatment. **a,d** Binary time series ($z(i)$) denoted 1 for activity and -1 for inactivity. **b, e** Integrated sequences obtained by cumulatively summing the previous binary series. **c,f** Log-log plots of the average fluctuation $F(n)$ at each scale as a function of scale (n). The α exponents reflect the slope of the regression lines.

were included in the models as response variables, while estimators from the full sequences were used as predictor variables. Age was also included as a predictor variable to control for its potential effect on behavioural complexity. Sheep identity and observation date were set as random effects in order to control for pseudoreplication and temporal variability in the daily sequences, respectively. As the distributions of the fractal exponents were non-normal, we transformed the values using the Johnson transformation in the ‘Johnson’ package (Fernandez 2014).

Relating to our main study aims, we then constructed a series of GLMMs to test whether these fractal properties differed between control and dewormed sheep before and after anthelmintic treatment. Response variables again included each of the scaling exponents measured, with the predictor variables in each model including experimental group (treated vs control), experiment period (pre- versus post-treatment), and their interaction; the interaction term was the main target of this study because only a subset of subjects were treated and we did not expect any pre-treatment variation in activity patterns. As above, sheep identity and date were both set as random effects to control for pseudoreplication caused by repeated behaviour sampling and for temporal variability. Again, a Johnson transformation was applied to the fractal exponents to normalize them before the analysis.

To test the significance of the interaction between experimental group and period, we compared the fit of the full model with that of a null model containing all predictor variables included in the full model except the interaction. This was conducted via likelihood ratio tests using the package ‘lmerTest’ (Zeileis and Hothorn, 2002). We report parameter estimates of these GLMMs only where significant interactions were first observed using the likelihood ratio test, as non-significant interaction terms would mean that the response variable does

not vary significantly relative to controls as a function of anthelmintic treatment.

We also tested whether the total duration, frequency and mean duration of activity and inactivity differed in the day-to-day sequences before and after treatment by constructing a series of similar models but including each standard behavioural measure as the response variable. In order to compare variation in standard activity measures before and after treatment between control and treated group, mixed-design ANOVA models were performed by including experimental period as a within-subject variable and group as a between-subject variable.

For all models we assessed deviations from the assumptions of residual normality and homogeneity, and found no clear evidence for violations. Alpha levels were set at 0.05 for all analyses.

3. Results

Accelerometry data suggest that our sheep were found to spend a mean \pm s.d. of $66.03 \pm 24.49\%$ of the day (range:13–89) and $18.30 \pm 8.58\%$ of the night (range: 1.97–57.48) active over the three experimental phases. These figures were similar to the activity budgets previously provided for domestic sheep (average time spent grazing and walking within 14.5 h of daylight: 88%, Aldezabal et al., 1999; average time spent grazing within 14 h of daylight: $64 \pm 22\%$, Pokorná et al., 2013), suggesting that accelerometry data provide an accurate representation of sheep activity/inactivity patterns. During the entire experimental period, the total time spent active was 6.59 ± 2.44 h (range: 0.002–10.66) per day and 2.19 ± 1.03 h (range: 0.24–6.90) per night.

At the onset of the pre-treatment period, the estimated intensity of strongylid infection ranged from 26.85 to 1631.70 EPG

(522.28 ± 464.71) in the control group, and from 31.94 to 2031.75 EPG (435.00 ± 491.81) in the treatment group. At the onset of the post-treatment period, the EPG ranged from 126.32 to 1538.46 (399.99 ± 405.90) in the control group, and was null in the treatment group.

The R²-SSR procedure demonstrated that the best scaling region lay between 2¹⁰–2¹⁴, 1024–16384 s or 17.1–273.1 min using DFA, and between 2⁸–2¹², 256–4096 s or 4.3–68.3 min using DFA_b. Therefore, we only calculated scaling exponents within these ranges of time scales for each DFA method, respectively (Appendix Fig. 3).

Fractal analyses showed that sequences of activity from domestic sheep exhibited long-range and antipersistent autocorrelation. Values of αDFA averaged 0.19 ± 0.08, suggesting that the original sequences produced are antipersistent fractional Gaussian noise (fGn) before integration [fGn: αDFA (0,0.5)]. This was confirmed by the fact that integrated sequences measured by DFA_b produced α values (1,1.5) characteristic of antipersistent fractional Brownian motion (fBm). A high correlation between the two DFA measures was observed (r = 0.75, p < 0.001) as theoretically predicted (αDFA ≈ αDFA_b - 1).

We found that values of αDFA_b estimated from the full sequences were significantly associated with the values calculated from the daily sequences (GLMM: αDFA_b, df = 38, t = 2.75, p = 0.009) and values of H_S estimated from the full sequences tended to be correlated with the values calculated from the daily sequences; H_S, df = 38, t = 2.02, p = 0.052). However, values of αDFA and FD_b from the full week-long sequences did not correlate with the daily values (GLMM: αDFA, df = 40, t = 0.67, p = 0.50; FD_b, df = 40, t = -0.03, p = 0.97). Correlations between fractal estimates calculated from daily and full sequences are shown in Fig. 2.

Between the pre-treatment and the post-treatment period, the difference in the averaged Hurst coefficients H₁ and H₂ depended on the experimental group (Fig. 3, Appendix Fig. 4). After treatment, the means of both H₁ and H₂ increased in the treated group (H₁ mean difference = 0.023; H₂ mean difference = 0.008) whereas both means decreased in the control group (H₁ mean difference = -0.006; H₂

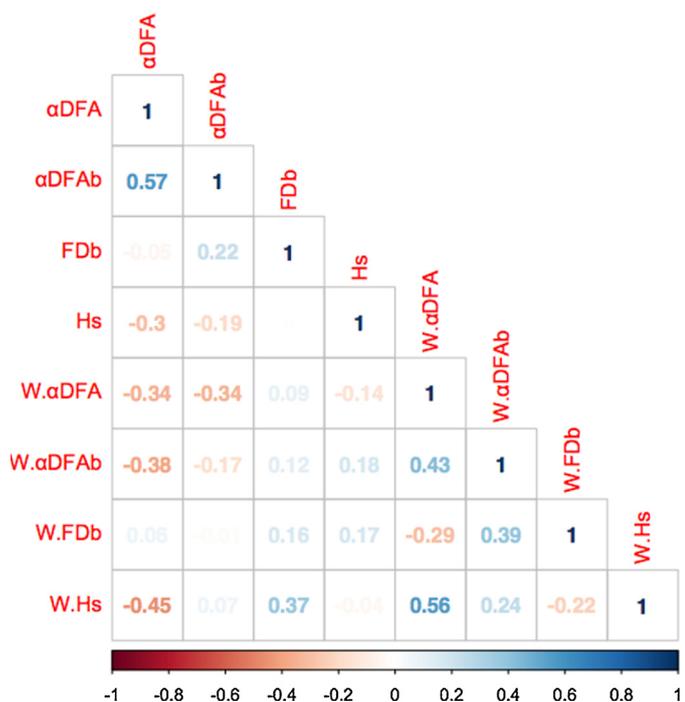


Fig. 2. Correlations between fractal estimates derived from daily activity sequences and from full, weekly sequences recorded during the pre-treatment period. “W” indicates coefficients calculated from the weekly sequences.

mean difference = -0.043) for values derived from daily sequences. Before treatment, the mean ± s.d. fractal estimates from daily sequences were: αDFA = 0.19 ± 0.08; αDFA_b = 1.27 ± 0.14; H_S = 0.87 ± 0.06; FD_b = 1.87 ± 0.08; H₁ = 0.44 ± 0.06; H₂ = 0.23 ± 0.10. The mean ± s.d. estimators after treatment were: αDFA = 0.19 ± 0.78; αDFA_b = 1.24 ± 0.08; H_S = 0.91 ± 0.06; FD_b = 1.54 ± 0.08; H₁ = 0.45 ± 0.06; H₂ = 0.21 ± 0.10.

Our models testing the influence of deworming on activity patterns showed a significant interaction between experimental group and period for each of αDFA, αDFA_b, H₁ and H₂, calculated from the daily sequences (likelihood ratio test: αDFA: χ² = 5.56, df = 1, p = .02; R² = 0.35; αDFA_b: χ² = 5.53, df = 1, p = .02; R² = 0.36; H₁: χ² = 8.30, df = 1, p = .004; R² = 0.38; H₂: χ² = 6.28, df = 1, p = .01; R² = 0.37), but we observed no such relationship for FD_b and H_S (likelihood ratio test: FD_b: χ² = 0.22, df = 1, p = .64; H_S: χ² = 1.16, df = 1, p = .28) (Table 1). The interactive effect between period and group for H₁ and H₂ demonstrates a significant variation in fractal estimates between control and dewormed sheep before and after anthelmintic treatment (Fig. 3).

In contrast, our analyses of the full, week-long activity sequences showed that treatment and period only interacted to influence variation in H₁ (likelihood ratio test: χ² = 4.76, df = 1, p = .03; R² = 0.57), although parameter estimation shows that this interaction may have only a marginal effect in the model (Table 2). Otherwise, we found no significant interaction for the remaining estimates concerning these sequences (likelihood ratio test: αDFA: χ² = 0.87, df = 1, p = .35; αDFA_b: χ² = 3.26, df = 1, p = .07; FD_b: χ² = 2.01, df = 1, p = .16; H_S: χ² = 3.61, df = 1, p = .06; H₂: χ² = 2.24, df = 1, p = .13). Age had no significant effect on fractal estimates in each of our models.

No significant interaction was found between experimental group and period for total time, frequency or mean duration of activity calculated over daily sequences (likelihood ratio test: Total time: χ² = 1.56, df = 1, p = .21; Frequency: χ² = 1.62, df = 1, p = .20; Mean duration: χ² = 0.64, df = 1, p = .42). Furthermore, comparisons between control and treated groups did not show any significant effects of anthelmintic treatment on the standard behavioural measures (ANOVA: Total time: F = 0.4, df = 1, p = .53; Frequency: F = 0.72, df = 1, p = .40; Mean duration: F = 0.38, df = 1, p = .54) (Fig. 4).

4. Discussion

In this study, we demonstrate using DFA that sheep behaviour sequences are characterized by long-range correlations, meaning that sequences of activity and inactivity do not fluctuate randomly through time, but depend on long-term activity events. Moreover, we show that the temporal organization of sheep activity varies with the strongylid infection status. The inconsistencies in our results depending on the exponents used, despite the significant correlations amongst them, highlight the importance of applying different fractal methods, and of averaging the resultant Hurst exponents as perhaps the most reliable, if conservative, estimate. While DFA remains a specific method for examining binary sequences of animal behaviour that may hold greater potential for diagnosis, taking the average of Hurst exponents derived from several fractal analyses may better reflect the degree of variation in complexity. Using this approach, our results indicate that sheep treated with anthelmintics exhibited a higher complexity (i.e. higher stochasticity) in both their daily and weekly activity sequences than parasitized sheep, suggesting that organizational patterns of their behaviour change with gastrointestinal parasite infection. This finding is in agreement with our predictions, and the generally observed complexity loss associated with pathology or stress.

Under normal conditions, complexity in behaviour is thought to reflect an optimal strategy to cope with heterogeneous internal or external perturbations. In animal movements, fractal-like patterns were proposed to have evolved because their super-diffusive properties increase the chances of encountering resources in a heterogeneous

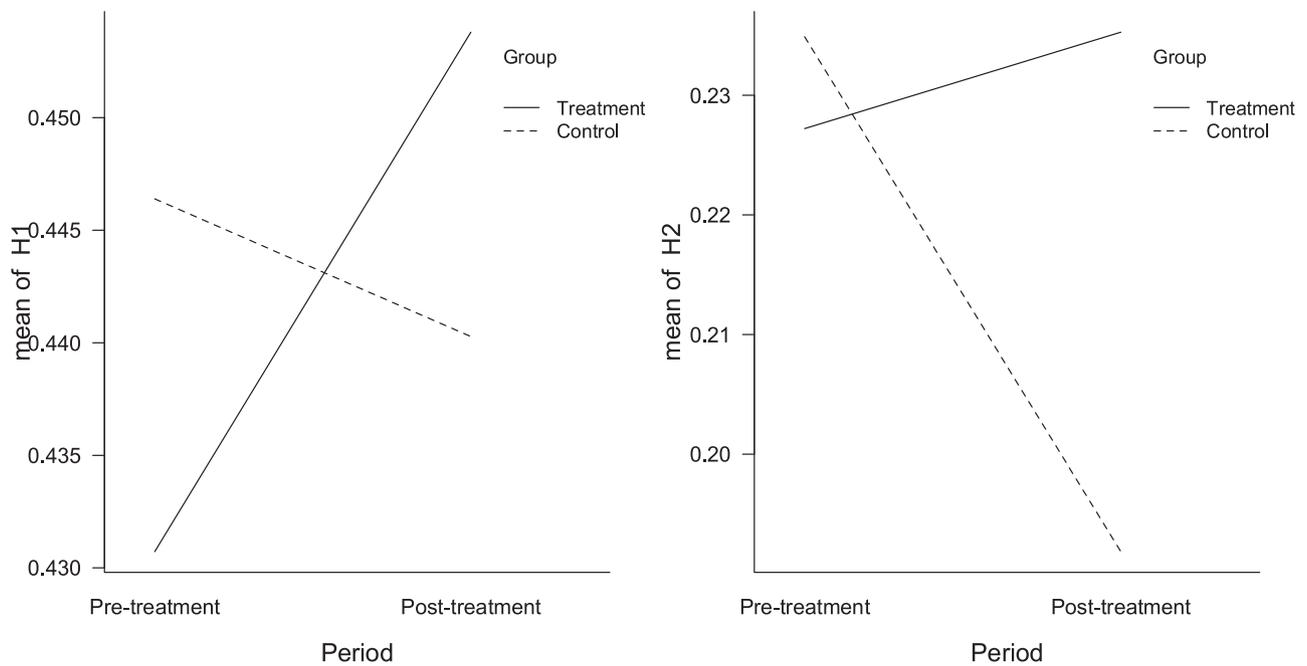


Fig. 3. Interaction between ‘treatment’ and ‘control’ group for H₁ and H₂, derived from daily activity sequences.

Table 1

Results of generalized linear mixed models (GLMMs) testing the effect of deworming on all fractal estimates from sheep daily activity sequences.

αDFA	Estimate	Standard Error	Confidence Interval	df	t value	p value
Intercept	-0.1787	0.2616	-0.7066 to 0.3488	34.65	-0.683	0.4992
Period (Pre- versus post-treatment)	0.3116	0.2261	-0.1445 to 0.7651	38.49	1.378	0.1762
Group (Treated versus control)	0.3688	0.205	-0.0568 to 0.7827	33.81	1.757	0.0807
Age	-0.0036	0.0347	-0.0750 to 0.0681	19.43	-0.105	0.9172
Period * Group	-0.4852	0.2047	-0.8890 to -0.0826	230.14	-2.371	0.0186 *
αDFA_b						
Intercept	-0.8199	0.2814	-1.3930 to -0.2496	27.88	-2.913	0.0069 *
Period (Pre- versus post-treatment)	0.5221	0.1993	0.1228 to 0.9208	41.93	2.63	0.0120 *
Group (Treated versus control)	0.5414	0.2335	0.0649 to 1.0122	29.40	2.32	0.0275 *
Age	0.0692	0.4033	-0.0136 to 0.1527	19.09	1.72	0.1024
Period * Group	-0.4858	0.2053	-0.8905 to -0.0817	230.41	-2.37	0.0187 *
H₁						
Intercept	-0.2063	0.2845	-0.7831 to 0.3692	30.29	-0.725	0.4739
Period (Pre- versus post-treatment)	0.128	0.2137	-0.3021 to 0.5575	37.93	0.599	0.5526
Group (Treated versus control)	0.3034	0.2285	-0.1635 to 0.7642	28.68	1.328	0.1946
Age	0.0153	0.0397	-0.0664 to 0.0975	19.19	0.385	0.7047
Period * Group	-0.5639	0.1943	-0.9467 to -0.1814	230.01	-2.902	0.0040 **
H₂						
Intercept	-0.5585	0.2774	-1.1213 to 0.0022	30.55	-2.013	0.053
Period (Pre- versus post-treatment)	0.461	0.2138	0.0306 to 0.8902	39.19	2.156	0.0373 *
Group (Treated versus control)	0.4937	0.2241	0.0365 to 0.9454	29.95	2.203	0.0355 *
Age	0.0449	0.0385	-0.0341 to 0.1247	19.14	1.167	0.2575
Period * Group	-0.5032	0.2002	-0.8981 to -0.1092	229.89	-2.513	0.0127 *

environment (Viswanathan et al., 2008). A significant body of evidence suggests that fractal movement patterns are produced by a large variety of organisms, ranging from bacteria (Ariel et al., 2015), insects like honeybees (Reynolds, 2007) and termites (Miramontes et al., 2014),

birds like albatrosses (Viswanathan et al., 1996), to mammals like spider monkeys (Ramos-Fernández et al., 2004) and even humans (Bertrand et al., 2007; Raichlen et al., 2014).

However, despite the considerable evidence for the existence of

Table 2

Results of generalized linear mixed models (GLMMs) testing the effect of deworming on averaged Hurst exponents from sheep weekly activity sequences.

H ₁	Estimate	Standard Error	Confidence Interval	df	t value	p value
Intercept	-0.2077	0.4296	-1.0930 to 0.6745	23.34	-0.482	0.0633
Period (Pre- versus post-treatment)	-0.0113	0.3868	-0.8693 to 0.8879	7.83	-0.029	0.9774
Group (Treated versus control)	0.1614	0.3657	-0.5793 to 0.9021	32.92	0.441	0.6619
Age	0.0546	0.0585	-0.0658 to 0.1761	18.66	0.933	0.3629
Period * Group	-0.9239	0.4015	-1.7838 to -0.1034	18.07	-2.301	0.0335*

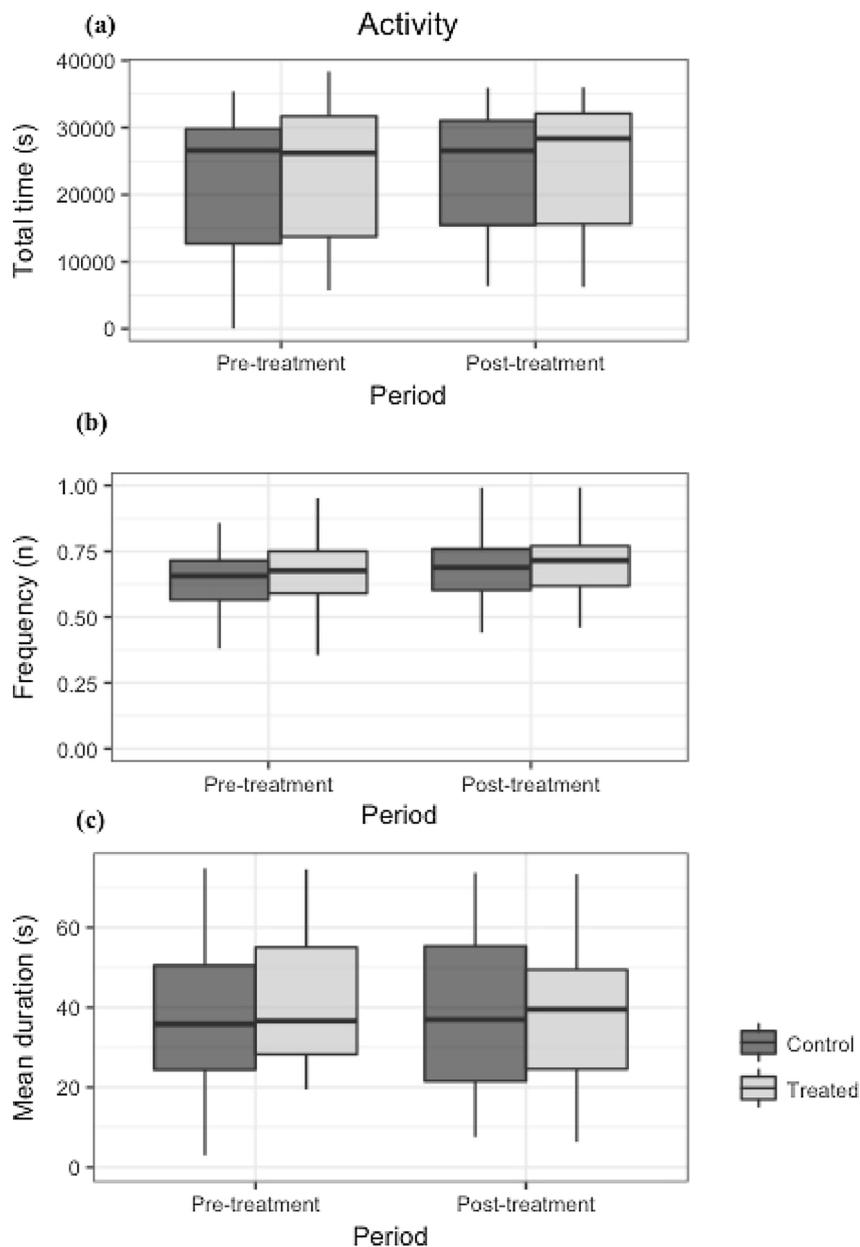


Fig. 4. Boxplots of total time (a), frequency (b) and mean duration (c) of activity calculated over daily sequences. Bold horizontal lines indicate median values, boxes reflect interquartile ranges, and whiskers reflect the minimum and maximum values that do not exceed 1.5 times the interquartile range from the median.

fractal patterns in animal behaviour, the underlying mechanisms regulating and/or disrupting them have not yet been established. It has been speculated that a neural network of interacting control nodes is responsible for scale-invariant locomotor patterns and that this endogenous control mechanism could exist across species. For example, Hu et al. (2007), reported that suprachiasmatic nucleus (SNC) lesion in rats completely suppressed fractal-like locomotor patterns over a range of specific time scales. Moreover, by associating movement patterns to neurobiological processes in mud snails, Reynolds et al. (2016) suggested that the production of fractal movement patterns can be driven by the temporal dynamics of their neuronal activity. Fractal fluctuations have been hypothesized to emerge from a network of biological oscillators, functioning across multiple locations and temporal scales, nonlinearly interrelated through feed-back loops, and the SNC would play a central role in the fractal organization of behavioural activity (Hu et al., 2007, see review in Pittman-Polletta et al., 2013).

While our study was not designed to investigate potential mechanisms, deviations from the theoretically optimal patterns may be the

direct result of a disruption of this network under pathological or stressful conditions. For example, energetic costs associated with gastrointestinal parasitism may be responsible for a dysfunction in the neurophysiological pathway controlling complex patterns (Ames, 2000; Tedford and McConkey, 2017). Strongylid infection in sheep can be characterized by diarrhoea, anorexia, anemia, dehydration and lameness (Lloyd, 2017). It can reduce host appetite and intestinal nutrient absorption, leading to an overall reduction in energy acquisition. Additionally, infected hosts have to allocate energy, for example, to tissue repair and to mounting an immune response. It is possible that the metabolic cost of parasitism causes an imbalance in the system mediating behavioural fluctuations. In some studies, a lack of food and subsequently energy has been related to the development of stereotypies, which are repetitive, unvarying behaviours without apparent function (Appleby and Lawrence, 1987; Terlouw et al., 1991; Bergeron and Gonyou, 1997). Typically performed by animals in unsuitable captive conditions, stereotypic behaviours are characterized by strikingly regular, periodic dynamics (Mason and Turner, 1993) and can

thus be viewed as representing an extreme loss in behavioural complexity. The emergence of this category of abnormal repetitive behaviours has been discussed as being a consequence of a perturbation of the central nervous system, or a coping response facilitating adaptation to stress or to an inadequate environment (Rushen, 1993; Mason and Rushen, 2006).

Equally, changes in complexity signatures may reflect a strategy to adjust to challenging conditions or compensate for their effects. Complexity loss may reflect an underlying allostatic overload, resulting from a potential trade-off between energy allocated to maintain the dynamics of components controlling variability in behavioural patterns and energy allocated to fight a disease or respond to other stressors. In this instance, as it has also been suggested regarding the expression of stereotypies (Pomerantz et al., 2012), dysfunction in the regulatory processes of the neurophysiological system and adaptive responses to better cope with adversity are not necessarily mutually exclusive mechanisms. Greater comprehension of the proximate mechanisms underlying variation in the irregularity of behaviour, and what their potential adaptive values might be, are now required to better understand the phenomenon of complexity loss. Understanding the causal factors implicated in the emergence of these patterns and why animals exhibit them will contribute to the fundamental knowledge of how behaviour is organized. Moreover, in the same way that stereotyped behaviours are widely used as indicators of poor welfare in captivity (Broom, 1991; Mason and Latham, 2004; Duepjan and Puppe, 2016), deviations from the normal, optimal range of complexity could be used as a means to assess animal welfare, particularly in the absence or before the onset of overt behavioural signs.

Our work brings further evidence that the fractal approach can be successfully used in assessing the well-being of domestic animals. Indeed, former applications of DFA to behavioural data were already able to reveal changes in behavioural complexity of stressed chickens (Maria et al., 2004; Rutherford et al., 2006), Japanese quail (Kembro et al., 2009a) and pigs (Rutherford et al., 2004). Other studies have shown an association between parasitic infection and variation in the complexity of behaviour among Spanish ibex (Alados et al., 1996), wild Japanese macaques (MacIntosh et al., 2011) and wild chimpanzees (Alados and Huffman, 2000; Burgunder et al., 2017), illustrating the potential utility of fractal analysis to distinguish between individuals that may be influenced negatively by parasitic infection.

Changes in terms of activity patterns and postures in relation to gastrointestinal parasitism have previously been demonstrated in cattle, with parasitized individuals displaying less frequent but longer episodes of lying and standing, and an increase in average duration of feeding behaviour (Szyszka et al., 2012; Szyszka and Kyriazakis, 2013). Here, more conventional analyses did not reveal any relationship between parasite infection and sheep activity patterns. Therefore, complementing behavioural measurements such as frequency and duration of activity to indices of complexity could have important diagnostic value and should be considered in future welfare research (Hocking et al., 2007).

Beyond the study of welfare in domestic animals, the methodology has shown promising avenues for application in toxicological studies (Motohashi et al., 1993; Alados and Weber, 1999; Shimizu et al., 2002; Nimkerdphol and Nakagawa, 2008; Augusiak and Van den Brink, 2016), for example to monitor contaminants in the aquaculture industry (Eguiraun et al., 2014), or to detect environmental pollutants (Lechelt et al., 2000; Seuront and Leterme, 2007). Finally, in wild animals, complexity loss was also observed, for example, in free-living penguins during experimental induction of increased circulating corticosterone (Cottin et al., 2014), suggesting utility in conservation, and even with attachment of certain larger-sized bio-logging devices (Meyer et al., 2015), with potential implications for technological considerations in study design.

This study is one of the very few to have applied fractal time-series analyses to bio-logged data. Considering the potential of using

advanced analytical tools combined with novel technologies to detect pathologies and/or potential production losses, it is our hope that this will stimulate further research along these lines. Indeed, further development of early-detection systems that might aid in the control of major threats to animal welfare, such as helminth infection in livestock production systems, could greatly benefit from increasing our understanding of behavioural organization and its variation with stress or disease.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.applanim.2018.05.014>.

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