Evaluation of the Open-Access Video Tracking Programme Swarm Sight to Evaluate Lethargy in a Ferret Model of Influenza Infection

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Abstract

As an animal model of influenza, ferrets are uniquely capable of displaying clinical signs of illness similar to those of human influenza virus infection. To quantify lethargy, we previously established video monitoring as a more sensitive method than the commonly used manual scoring methodology for ferrets infected with influenza virus. Video monitoring is simple to set-up, but its adoption by other laboratories is restricted by the need to purchase costly commercial software, EthoVision® XT, to analyse activity. To broaden the use of video monitoring method in ferrets, we assessed Swarm Sight, a free open-access programme, for analysing activity changes in ferrets infected with seasonal influenza A(H1N1), A(H1N1) pdm09, A(H3N2) and B virus. Swarm Sight could differentiate between the various levels of lethargy associated with the infection of different influenza virus subtypes to a similar degree to EthoVision® XT. However, one major limitation of Swarm Sight is that it does not permit high throughput analysis, which considerably increases the time required to process video clips from experiments involving large numbers of ferrets. Despite this limitation, the open-accessibility and comparable results to EthoVision® XT make Swarm Sight a good alternative for researchers interested in using video monitoring to measure lethargy in ferrets.

Keywords: Influenza; Ferret; Activity; Lethargy; Video; Virus

Introduction

Influenza is a highly contagious viral disease that causes high mortality and morbidity [1]. Ferrets are considered the ideal model for studying aspects of influenza infection such as viral pathogenicity, viral fitness and clinical effectiveness of different antivirals [2-4]. Among the different animal models, the ferret is the only animal that displays clinical signs similar to those of humans following influenza infection. A majority of clinical signs, such as weight loss and elevated body temperature can be measured accurately, but other more subjective clinical signs, such as lethargy, are more challenging to measure. To measure lethargy in ferrets infected with influenza virus, we have established a simple protocol using video monitoring to analyse ferret activity [5]. In comparison to the manual scoring method, video monitoring is more sensitive at detecting minor changes in ferret activity and is not subject to operator bias [5]. The video monitoring method involves a filming step followed by video analysis. The physical set-up for filming is relatively simple and inexpensive. However, the major limitation for the wide application of this video monitoring method is the cost associated with the purchase of video analysis software, such as EthoVision® XT as used in our previous study. To broaden the use of video monitoring to assess ferret lethargy, a more affordable video analysis programme is needed. Swarm Sight is a free open-access software programme which was previously validated for assessing behavioural changes in bees, wild birds and insects [6]. In this study, we sought to investigate the use of Swarm Sight, compared to EthoVision® XT, to assess changes in activity level in ferrets infected with influenza A or B viruses.

Materials and Methods

Ethics statement

Experiments using ferrets were conducted under the approval of the CSL Limited/Pfizer Animal Ethics Committee (project license number 868) or Melbourne University Animal Ethics Committee (project license number 1313040) in strict accordance with the Australian Government, National Health and Medical Research Council Australian code of practice for the care and use of animals for scientific purposes (8th edition).

Ferrets

Outbred 6 to 8 month old male and female ferrets (*Mustela putorius furo*; Animalactic Animals & Animal Products Pty Ltd, Victoria, Australia) weighing 600–1543 g were used. Serum samples from ferrets were tested by haemagglutination inhibition assay against the reference strains A/California/7/2009 (H1N1) pdm09, A/Victoria/361/2011 (H3N2), B/Wisconsin/1/2010 (B/Yamagata-lineage) and B/Brisbane/60/2008 (B/Victoria-lineage) to ensure seronegativity against currently circulating influenza subtypes and lineages. The allocation of ferrets to different groups (n=3-12 per group; total = 22 ferrets) was randomised. Ferrets were housed individually in high efficiency particulate air filtered cages with *ad libitum* food, water and enrichment equipment.

Virus infection of ferrets

Ferrets were anaesthetized [50:50 mix of (100 mg mL^{-1}): Ilium Xylazil (Xylazine; 20 mg mL^{-1})] and infected with influenza seasonal A/Mississippi/3/2001 (H1N1), A/Perth/265/2009 (H1N1) pdm09, A/Fukai/20/2004 (H3N2) or B/Yamanashi/166/1998 (B/Yamagata-lineage)

respectively. For influenza A(H1N1) pdm09 and B viruses, ferrets were infected by intranasal instillation with 10^5 TCID$_{50}$ (median tissue culture infectious dose) of MDCK (Madin-Darby canine kidney (MDCK; ATCC® CCL-34™)-propagated viruses. For influenza seasonal A(H1N1) and A(H3N2) viruses, ferrets were infected by contact transmission whereby experimentally infected donor ferrets were co-housed with naïve recipients for 48-hours to initiate the infection. Nasal washes were collected daily from sedated ferrets (intramuscular injection of Xylazine at 5 mg kg^{-1}) by instilling 1 mL of sterile PBS into one nostril and allowing the liquid to flow out of the other nostril into a collection tube. Titres of infectious virus in the nasal washes were quantified by a viral infectivity assay as described by Reed and Muench [7].

Video filming of ferrets

Daily video monitoring of ferret activity was carried out as previously described [5]. Ferrets were removed from their respective cages and placed into a 400 litre box and their activity was recorded for 1 minute (Figure 1). Between the filming of each ferret, the box was cleaned with 80% (v/v) ethanol. To determine the baseline activity of each ferret, measurements were taken for 5 consecutive days prior to viral infection. The filming sequence of individual ferrets in all groups was randomly assigned and filming was carried out daily at approximately the same time each day (between 8 to 9 am).

Activity analysis with EthoVision® XT

Activity levels were measured using the motion analysis programme, EthoVision® XT 10.0 (Noldus IT, Netherland) as previously described [5]. Video files (AVI. format; converted from MTS. format) were uploaded into EthoVision® XT and the activity level of ferrets was measured by the software as a function of pixel change.

Activity analysis with Swarm Sight

Activity was measured using the free open-access motion analysis programme, Swarm Sight [6]. Video files (AVI. or MTS. format) were individually uploaded and activity analysed using Swarm Sight. Within the activity settings, 'motion threshold' was adjusted to 29 to minimise background noise and 'motion contrast' and 'speed' were adjusted to 1X and 100%, respectively. The 'region of interest' for analysis included the whole box to capture all movements of the ferret. The recorded video was analysed for 1 minute and the number of changed pixels for each frame was tabulated by the Swarm Sight programme. A daily mean pixel change per frame (a total of 1684 frames) was then calculated for each ferret.

Statistical analysis

Unpaired t-test was used to compare between 'low' and 'high' activity for Swarm Sight and EthoVision® XT. Mann-Whitney U non-parametric test was used to compare day-to-day differences in mean activity between the different groups. A P-value *of* <0.05 was considered statistically significant.

Results

Assessment of Swarm Sight to analyse ferret activity

To assess the preliminary suitability of Swarm Sight to analyse ferret activity, 20 videos depicting 'low' and 'high' ferret activity (as determined by EthoVision® XT) were analysed using Swarm Sight. As shown in Figure 2 Swarm Sight was able to clearly discriminate between the two levels of activity where the relative fold difference between the mean levels was similar to that measured by EthoVision® XT (Swarm Sight: 2.3-fold versus EthoVision® XT: 2.2-fold). These data demonstrated that Swarm Sight could be used to analyse videos of ferrets using our established filming set-up and produce activity data comparable to that determined from EthoVision® XT.

Assessment of Swarm Sight to analyse ferret activity post influenza infection

To further assess whether Swarm Sight was a suitable programme to analyse ferret activity following influenza virus infection, we analysed a series of video recordings of ferrets infected with different influenza viruses. Swarm Sight and EthoVision® XT analyses were carried out on the same set of videos. The influenza viral shedding profile for each group of ferrets infected with a particular influenza virus is provided in Figure S1.

Ferrets infected with influenza A(H1N1) pdm09 viruses experienced low activity from days 2 to 6 post-infection (pi), before activity began to increase after day 6 pi and returned to above baseline levels on day 11 pi (Figure 3A). The activity level data provided by EthoVision® XT and Swarm Sight showed a high degree of similarity. For ferrets infected with the influenza seasonal A(H1N1) viruses, both EthoVision® XT and Swarm Sight showed that infected ferrets had a reduction in activity level on day 1 pi and reached a nadir on day 4 pi, before gradually returning to near baseline levels of activity on days 9 and 11 pi (Figure 3B). Although there was slightly less similarity between these data points than that observed for A(H1N1) pdm09, there was no statistical difference between the results from
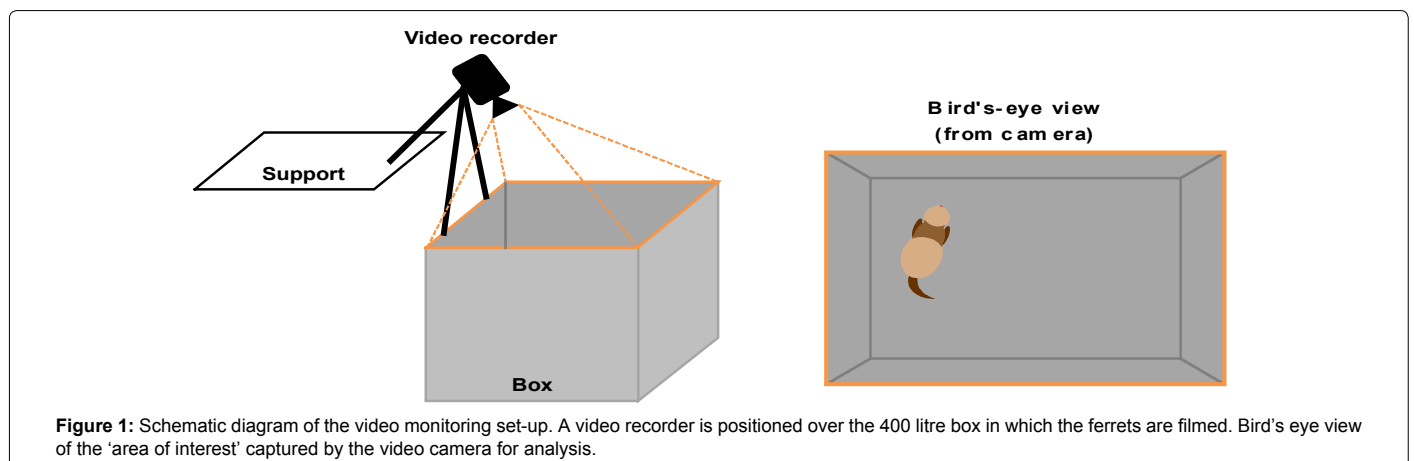

Figure 1: Schematic diagram of the video monitoring set-up. A video recorder is positioned over the 400 litre box in which the ferrets are filmed. Bird's eye view of the 'area of interest' captured by the video camera for analysis.
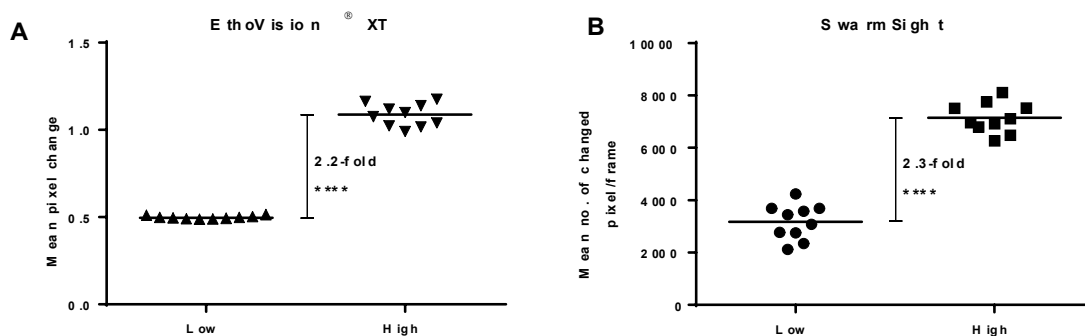
Figure 2: Assessment of Swarm Sight to analyse ferret activity. Analysis of the videos of ferrets with either 'low' or 'high' activity using (A) EthoVision®XT and (B) SwarmSight. Each data point represent a single video (n = 10/condition). Line represents mean value. 'Low' versus 'high' activity: **** P < 0.0001, unpaired t-test.
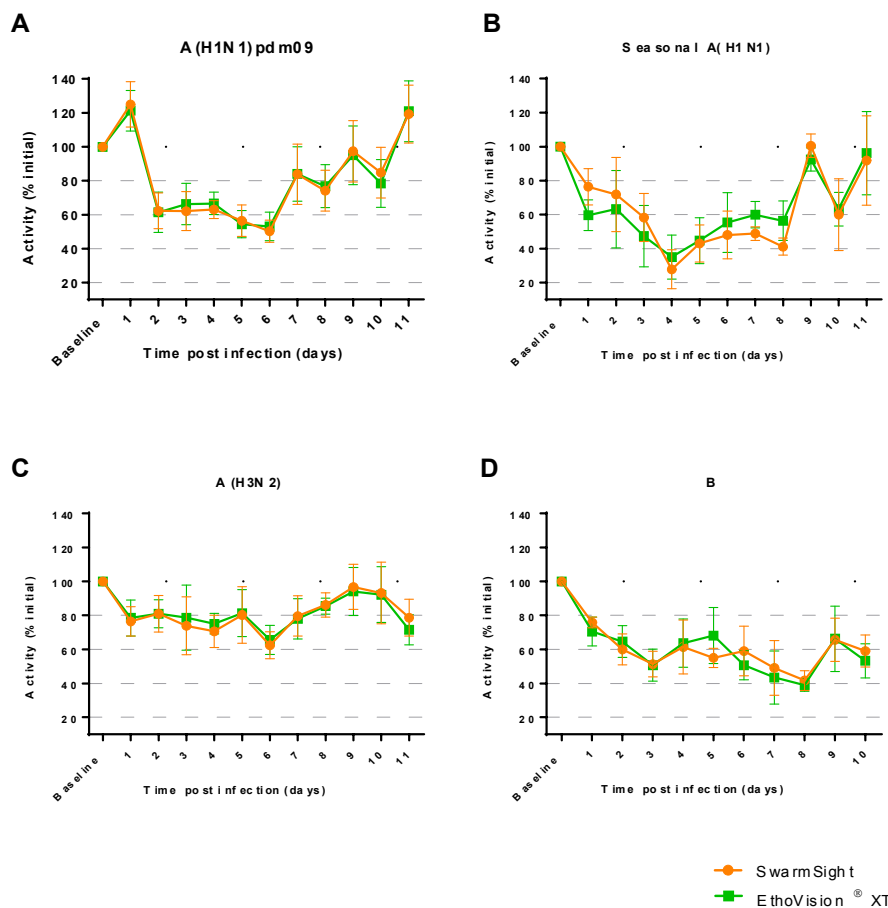

Figure 3: Activity level changes of ferrets infected with influenza viruses. (A) Seasonal influenza A(H1N1) (n=3), (B) A(H1N1)pdm09 (n=12), (C) A(H3N2) (n=4) and (D) B viruses (n=3). Daily videos of infected ferrets were analysed with Etho Vision® XT and Swarm Sight and compared to a 100% baseline activity level (dotted line) calculated prior to infection. Data shown are mean ± S.E.M.

the two programmes (Figure 3B). For influenza A(H3N2) infection, data from both Swarm Sight and EthoVision˚ XT again shared high similarity, showing that infected ferrets had a reduction in activity level from days 1 to 6 pi before returning to near baseline level on day 9 pi (Figure 3C). Ferrets infected with influenza B viruses experienced a reduction in activity level starting from day 1 pi but in contrast to ferrets infected with influenza A virus, the activity of influenza B virus infected ferrets remained below baseline levels until the end of the experiment (Figure 3D). Activity of ferrets as determined by Swarm Sight was slightly different to that from EthoVision˚ XT on day 5, 6 and 7 pi, although no statistical differences were detected (Figure 3D). Videos of days with differences in activity between EthoVision˚ and Swarm Sight were verified visually but no unusual ferret movements were noted.

Discussion and Conclusion

The introduction of computational analysis allows us to detect and analyse behavioural changes of animals to specific stimuli or pathogens with greater sensitivity. In this study, we evaluated the use of Swarm Sight, a free open-access programme, to analyse ferret activity using our previously described video monitoring set-up [5]. The methodology was originally established using a commercial software programme (EthoVision® XT), however due to the large cost of purchasing the software we were interested in assessing the applicability of a free open-access software programme, Swarm Sight, as an alternative to analyse ferret activity. Our study revealed that ferret activity data derived from Swarm Sight is highly comparable to that from EthoVision® XT. Although some differences in activity level output for the two programmes were observed on some days, both showed very similar trends, peaks and signs of recovery after influenza virus infections in ferrets. In addition to being a free open-access programme, Swarm Sight is easy to use and requires less training compared to the more sophisticated EthoVision® XT software. Importantly, Swarm Sight is also able to analyse videos in different formats taken directly from the video camera (AVI. and MTS.) compared to EthoVision® XT which requires videos to be converted to AVI format for analysis.

Several limitations with Swarm Sight were identified during our comparative analyses of ferret activity using our video monitoring set-up (Table 1). Firstly, because batch analysis is not permitted, analysis of a large number of videos becomes a more laborious process. For example, a typical experiment in our facility would involve 20 ferrets filmed for 5 days prior to infection (baseline) and 11 days pi, resulting in a total of 320 videos. Using Swarm Sight, it can take up to 2-3 days of hands-on-time to load and run the videos individually through the programme followed by data compilation. In contrast, analysis is more rapid using EthoVision® XT, which requires only 30 minutes of hands-on-time to load the videos and the analysis process can be run independently over a 24 hour period using pre-set parameters. Secondly, unlike EthoVision® XT, parameters such as time frame and frame rate cannot be adjusted in Swarm Sight which limits standardisation between video analyses. Additionally, manipulation of the 'area of analysis' is greatly limited in Swarm Sight compared to EthoVision® XT, where the latter allows users to more accurately define the area in which the activity levels will be measured. Additional analyses which are useful features of EthoVision® XT, such as heat-map visualisation of activity or measuring distance

travelled by the ferret are also not available in the version of Swarm Sight used in this study. Given that Swarm Sight is open-access and simple to use, it is more suitable for studies involving fewer ferrets or for laboratories beginning to do this sort of analysis. However, for larger scale experiments, researchers may still want to opt for the ease and additional features of EthoVision® XT.

To date, manual observation is the most widely used method for measuring ferret activity in influenza infection studies [8-10]. However, this method requires trained personnel to detect subtle changes in ferret activity, which can be particularly difficult in ferrets infected with seasonal human influenza viruses, which generally cause a mild disease. For example, Huang et al. [9] used manual scoring and reported no significant differences in ferret activity between seasonal A(H1N1), A(H1N1) pdm09, A(H3N2) and B influenza virus infections. However, using the video monitoring method and analysing videos with EthoVision® XT and Swarm Sight, we were able to identify clear reductions in activity for all ferrets, infected with influenza viruses. Of the four influenza types/subtypes analysed, seasonal A(H1N1) virus caused the greatest reduction in ferret activity of 70% on day 4 pi, compared to 60% on day 8 pi for influenza B, 50% on day 6 pi for A(H1N1) pdm09 and 37% on day 8 pi for A(H3N2) viruses. Nonetheless, we only assessed a single virus for each subtype and thus careful interpretation is warranted as the data may not be a representative for the respective subtypes. Also of interest was that ferrets infected with influenza B virus showed an extended period of decreased activity levels compared to influenza A viruses. Currently, available data on the effect of influenza B viruses on lethargy are limited, thus our preliminary findings warrant further study with other influenza B viruses, in particular viruses from both the B/Yamagata- and B/Victoria-lineages to see if this finding is consistent of all influenza B viruses. From an animal welfare perspective, video monitoring can also be a more definitive way to monitor changes in animal activity in conjunction with other parameters such as weight loss, temperature, hydration level and overall body condition. Not only does it better inform researchers and veterinarians on the overall well-being of animals on trial, but the video data can be stored for retrospective analyses to improve animal/experimental recording.

Overall, the video monitoring method is a more sensitive methodology than manual observation for determining the activity level changes in ferrets infected with influenza viruses. As lethargy is

Features		SwarmSight	EthoVision® XT
Accessibility/Cost		Open-access (Free)	Commercial product (~USD$5850)
Easy to use		Yes	Yes
Batch analysis		No	Yes
Ability to customise analysis	**Time range of analysis[a]**	No	Yes
	Area of interest[b]	Single setting: Rectangle	Multiple settings: Rectangle, open- and closed-polyline, ellipse, three-point circle
	Adjustment of frame rate[c]	No	Yes
	Heat-map	No	Yes
	Other measurements (e.g. distance moved)	No	Yes
	Ability to save video files in a designated folder	No	Yes
Video format required		Ability to analyse both AVI. And MTS. Format	Videos must be converted to AVI. Format

[a]Analysis can be programmed to start, pause or terminate at specific time-points of a video
[b]Specific regions of the video to be included in the analysis
[c]Frame rate of videos can be adjusted and specified for analysis

Table 1: Feature comparison between SwarmSight and EthoVision® XT.

an important parameter for assessing virus pathogenicity [2-4] and antiviral effectiveness [5] in a ferret model of influenza infection, it can be anticipated that the validation of Swarm Sight as a more affordable alternative will encourage more researchers to adopt video monitoring as the method of choice for measuring lethargy in a ferret model of influenza infection.

Competing Interests

No competing interests declared.

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Acknowledgement

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