Ref. Ares(2023)3511169 - 21/05/2023

VETBIONET

Veterinary Biocontained facility Network for excellence in animal infectiology research and experimentation

Deliverable D9.5

Validation of integrated telemetry and behavioural monitoring

Due date of deliverable: 72

Actual submission date: 72

Start date of the project: March 1st, 2017 **Duration:** 72 months

Organisation name of lead contractor: WBVR

Revision: V1

Dissemination level	
Public	**X**
Confidential, only for members of the consortium (including Commission Services)	
Classified, as referred to in Commission Decision 2001/844/EC	

Table of contents

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°731014

1 Summary

Changes in animal behaviour and physiological parameters are indispensable information on onset and progress of infectious diseases and valuable parameters in pathogenesis studies, treatment studies and vaccine studies. Complementing the current practice of repeated clinical observation with low frequency, continuous information on changes of behaviour or physiology provides more in-depth analysis in the dynamics of infection and protection. A range of different technical approaches have been addressed in the scope of animal species used in the context of VetBioNet research and transnational access studies. Behaviour was addressed by video-assisted analyses, by use of activity sensors or by a combination. The advantages of these systems in studying infections have been shown in this project in various mammalian species, avian species and fish. However, also drawbacks like difficulties identifying individual animals, failure in long term recording (over weeks, 24/7), the data management of huge data sets and the necessity of specialized data analysis became evident and progress was made by combining users' experiences and needs with the expertise of technological developers. These are major achievements for the field and necessary knowledge has been developed to implement the technology in specific studies on short term. However, implementation of these techniques as a regular, routine read-out in infection studies stays difficult due to the high extra work load and the necessary high expenses. Telemetric monitoring of physiological changes was limited to continuous or logged body temperature recording and analyses. Commercially available systems have been tested in various farm animal species and their robustness and reliability as well as high information value for interpretation of infection studies have been shown. Unfortunately, these sensors have to be implanted subcutaneously or intraperitoneally with invasive surgical procedures. Although no complications after implantation have been reported, the invasive procedures are considered to impair animal welfare. The usefulness of the obtained data to improve animal welfare during an infection study and the effect of the surgical procedure have to be considered. In summary, we performed successful behavioural monitoring in each group and physiological monitoring in almost all the animal groups. Although the studies were quite successful, many optimization steps still need to take place to better record the data, retrieve all desired parameters, and understand the acquired new data of each different animal species.

The results of behaviour and temperature measurements were proven useful across the multiple agents and species to assess and monitor infectious diseases. The continuous instrumented monitoring of behaviour and physiology brought new findings and insights that were easily missed before with clinical observations. However, optimization and standardization are still needed for each animal model and to define threshold values for the humane endpoint.

<u>VetBioNet partners involved</u>:

WBVR, Noldus, EMC, INRA-IERP, INRA-PFIE, ANSES, ANSES (Nancy), APHA, TPI, FLI, INIA, IZSVe, MRI, IRTA, AU

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°731014

2 Introduction

Animal behaviour research has developed as an indispensable information source in experimental studies in cognition and psychology research, in experimental pharmacology research and also in research in animal welfare and health. Animal experimental studies examining infectious diseases in farm animals (mammalian, avian, fish) and especially, those studying infections with highly virulent pathogens, which have to be performed in highly contained animal facilities and study microbiological parameters like local or systemic pathogen presence in the course of the disease and host parameters related to clinical disease signs or systemic immunological or inflammatory changes. Behaviour observations are an essential part of the clinical evaluation in such infection study, but often due to practical concerns, behaviour recordings are limited to short-term observations once or twice a day.

Animal experimental research in pharmacology and psychology have shown that more detailed behaviour analysis and long-term behaviour analysis led to a better interpretation of outcomes of studies and achievement of study objectives. Sophisticated techniques studying laboratory animal behaviour and other physiological responses over time and have been developed and successfully used. The transfer of this knowledge and expertise to the infection disease research in farm animals and also in laboratory animal models can provide substantial additional information to identify onset of disease at an earlier time-point, to detect sub-clinical disease and also cover the time outside regular observation moments which may be instrumental to identify the development of infectious disease and avoid severe animal suffering by the application of humane endpoints.

Socially housed (farm) animals actually require individual marking for robust behavioural monitoring. Measurements of animal behaviour such as changes in activity or changes in the amount of time spent can indicate health issues. Developments in sensor technology allow for (relatively) large-scale data gathering, using for example wearable accelerometers. However, often these systems are developed in animal welfare research for use over a few of hours during a specific measurement, and are not always suitable for long-term use (several weeks) in groups of infected animals. Adaptation of current systems and innovation by system development should be useful to detect behaviour and activity in a less time-consuming and more consistent manner than clinical checks by personnel and contribute to validation of experimental models of infectious diseases. Combined with primary scientific read-outs (virological, immunological and pathological data) these might also be translated to humane end point criteria to prevent animal suffering beyond the scientific end point. The objective of this deliverable was to improve current hardware and software systems to be experimentally applied in models of infectious diseases and to define and select physiological/behavioural hallmarks of the developing disease and, finally, to inform objective humane endpoints.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°731014

3 Results

3.1 Innovative monitoring methods and tools: technical perspective

3.1.1 Tools to measure behaviour

This paragraph gives an overview of the tools used in most of the pilots.

3.1.1.1 Video recording and annotation (Viso and The Observer XT)

Within WP9, Noldus' multi-room video recording tool Viso has been used to capture in detail animal behaviour with IP cameras. Recordings can be scheduled in advance or manually controlled by a user. The video recordings are imported into Noldus' scoring and analysis tool The Observer XT (Fig. 3-1). Once an ethogram has been defined one can have each observation manually annotated by a human expert based on the behaviour of the animal(s) in the video (system used by VetBioNet partners: Noldus, ANSES),

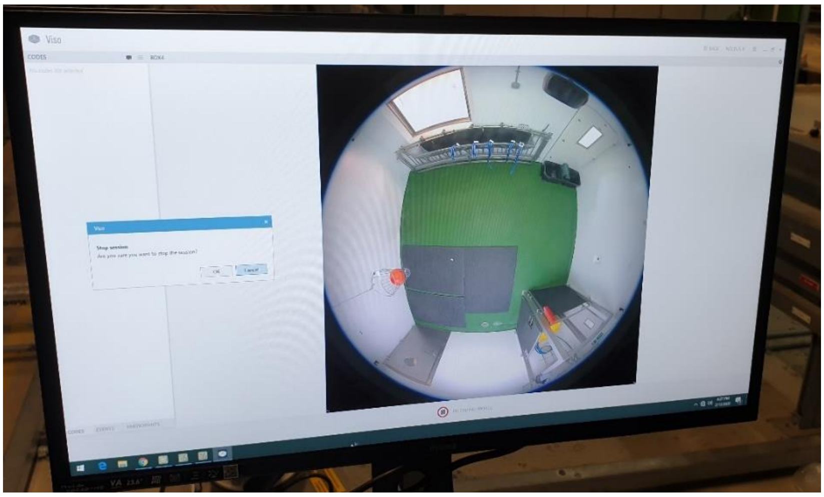

Figure 3-1 The animal room forms the arena for behavioural analysis

3.1.1.2 Video tracking (EthoVision XT)

Within WP9, automated recognition of behaviour in fish, cats and pigs was performed using EthoVision XT 16, provided by Noldus Information Technology. EthoVision XT has been on the market since 1993 and is continuously further developed based on the latest innovations in computer vision and AI technology. As a one-stop solution for behavioural analysis it offers users the ability to record video data, track the movement and postures of animals, and visualize the resulting behavioural findings. A wide variety of options is available for behavioural parameters for analysis, such as: Movement tracking; Activity and mobility analyses; Behaviour recognition; Data visualization and multiple unmarked subject tracking.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°731014

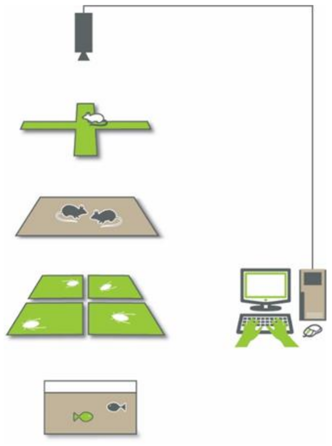

Figure 3-2 Schematic representation of an EthoVision XT setup; a camera is typically placed above an arena in which the experiment will take place. The resulting video image is collected and further analyzed on a PC

Core features of the EthoVision XT software package:

- Plug-and-play solution
- Intuitive user interface
- Live tracking and tracking of recorded videos
- Tracking up to 100 arenas and 16 animals per arena
- Import and synchronization of external data
- Analysis of individuals and groups of animals

System was used by VetBioNet partners: Noldus, WBVR, ANSES, .

3.1.1.3 *Individual color-based tracking (used at VetBioNet partners: Noldus, ANSES)*

EthoVision XT can be used to analyse the behaviour of individuals, both solitary or within a group, and analysis on the group level. When looking at individual animals within a group, color markers are used to distinguish individuals from each other (see for example figure 3-3).

Figure 3-3 . Example of the use of coloured markers in EthoVision XT. Each fish is equipped with a colour marker on the dorsal fin. These markers are then registered to virtual subjects within the software, by selecting the colours that should be recognized

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement N°731014

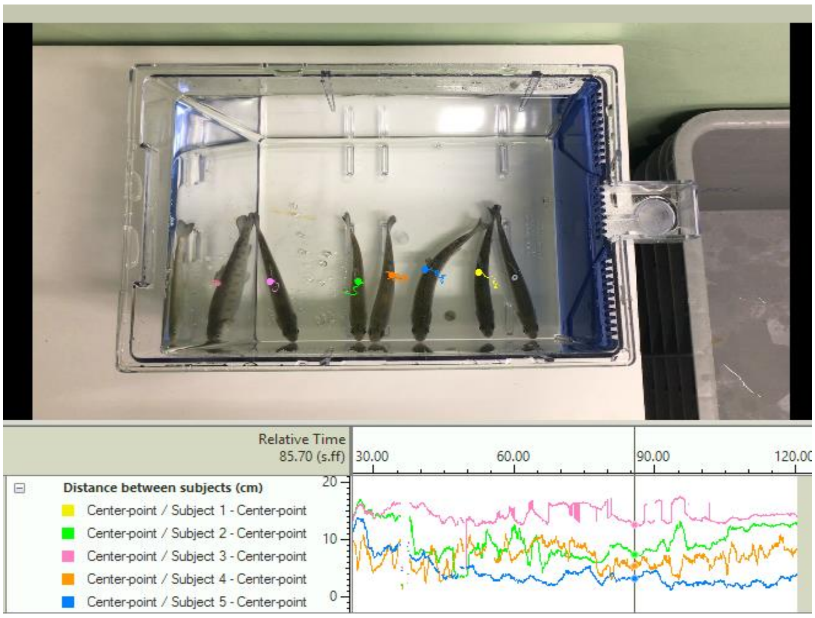

Figure 3-4 Example of the results based on individuals with color markers in EthoVision XT

This color marker is not only used to discriminate individuals, it also serves as reference point from which behavioural data is derived, based on 2D location and time. Using these measurements, EthoVision XT can calculate a wide variety of behavioural parameters (Fig 3-4).

System was used by VetBioNet partners: Noldus, IZSVe, INRA-IERP

3.1.1.4 Automated analysis of behaviour

Within VetBioNet, two types of analysis were performed on fish, one aimed at measuring the behaviour of individuals in a group and one aimed at measuring group level behaviour. In cats and pigs, a similar analysis of group level behaviour was conducted.

Individual rainbow trout could be tracked based on color markers attached to the animals, here in the form of colored beads attached to the dorsal fin. From these animals a variety of behavioural measurements were analyzed, including the following:

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°731014

Total distance moved; Velocity; Time spent (not) in movement; Distance between subjects; Time spent (not) in proximity of other subjects; Direction (heading); Location in space (Fig 3-5).

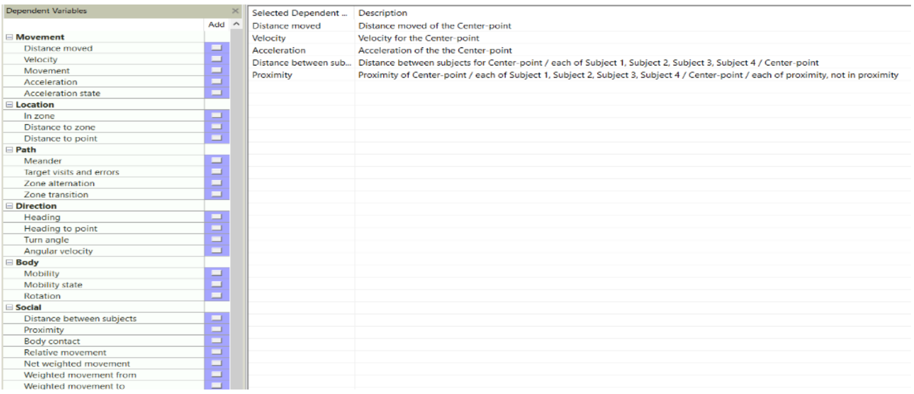

Figure 3-5 Examples of behavioral parameters that can be analyzed in EthoVision

Total distance moved, velocity and time spent (not) in movement are all measures of locomotor activity. Locomotor activity is often used as a proxy for wellbeing, as both infectious disease and stress have profound negative effects on these measures. Distance between the subject and time spent in proximity to other subjects are both measures of sociability. Like locomotor activity, sociability is negatively affected by pathogens and stress, placing the animal in a state of social withdrawal. Changes in the swimming direction and location in space are both indicators of swimming patterns, which can be related to the wellbeing of fish.

For example, the location of the animal can reveal thigmotaxis (i.e. the tendency to stay near the walls), which is a sign of stress.

At the group level, the behaviour of sea bass, cats, and pigs were analyzed. Here, locomotion was assessed using the activity analysis function of EthoVision XT. The activity analysis function detects changes in pixel gray value (see for example Fig. 3-6). With this, an estimate of the movement of animals can be made. This estimate is based on the whole experimental setup, meaning that all movement of the entire group is summed. As a measurement of locomotor activity, the activity analysis of EthoVision XT can also be used as a measurement of wellbeing, but now on the level of the whole group of interacting animals.

System was used by VetBioNet partners: Noldus, ANSES, APHA, TPI, WBVR

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement N°731014

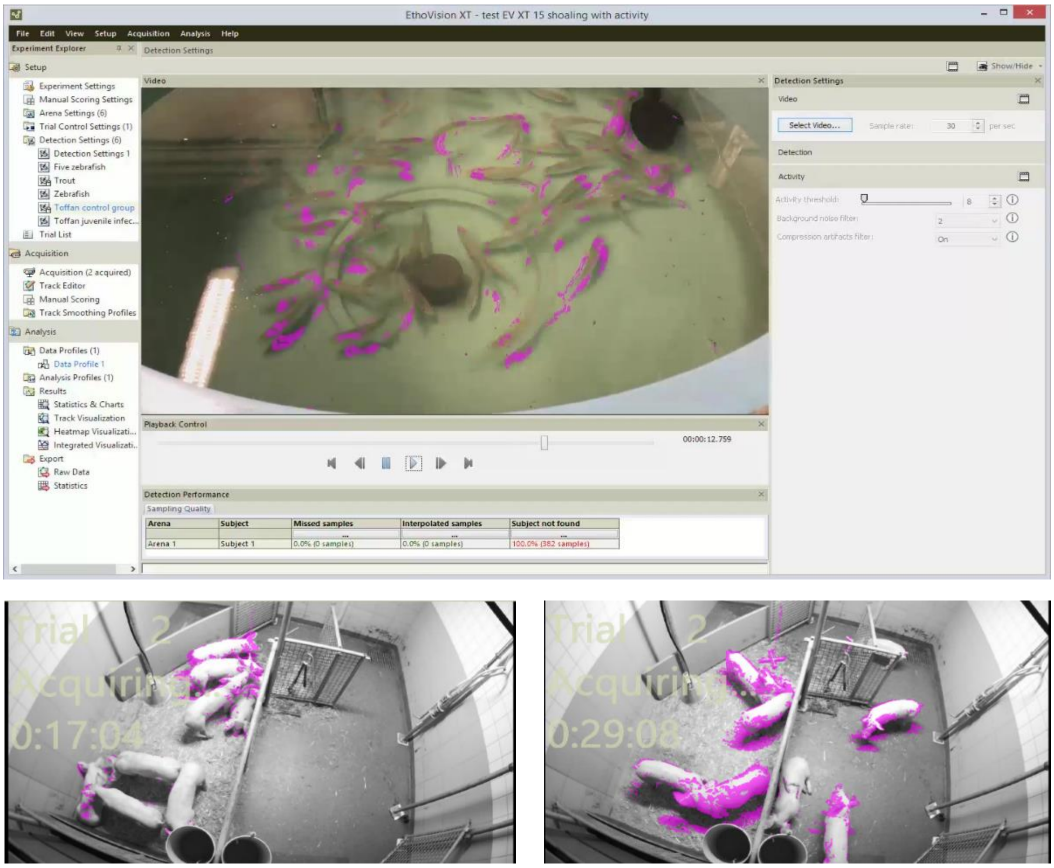

Figure 3-6 Figure 4. Examples of the activity analysis in EthoVision XT. Changes in pixel gray value above a predetermined threshold are shown in purple. Activity is measured as a single summed value over the whole group. Top: Fish, bottom left: moderately active pigs, bottom right: highly active pigs.

3.1.1.5 Ultrawideband tracking (TrackLab)

Noldus developed the TrackLab™ system for research into the activity, behaviour, welfare, and health of livestock animals. As depicted in Figure 6, sensor data is collected using an electronic tag mounted on the animal, either using a neck collar or a backpack for small birds, equipped with multiple measuring devices such as accelerometer and gyroscope. Animal positioning (timeseries of x,y data per animal) is based on ultra-wideband (UWB) radio communication between tag (on animal) and anchors (sensors mounted on wall or ceiling).

While information from these types of sensors is beneficial for automatic behaviour recognition, it merely provides information about the relative movement of an animal. Using the location of the animal as additional input source can improve the performance of the behaviour recognition.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°731014

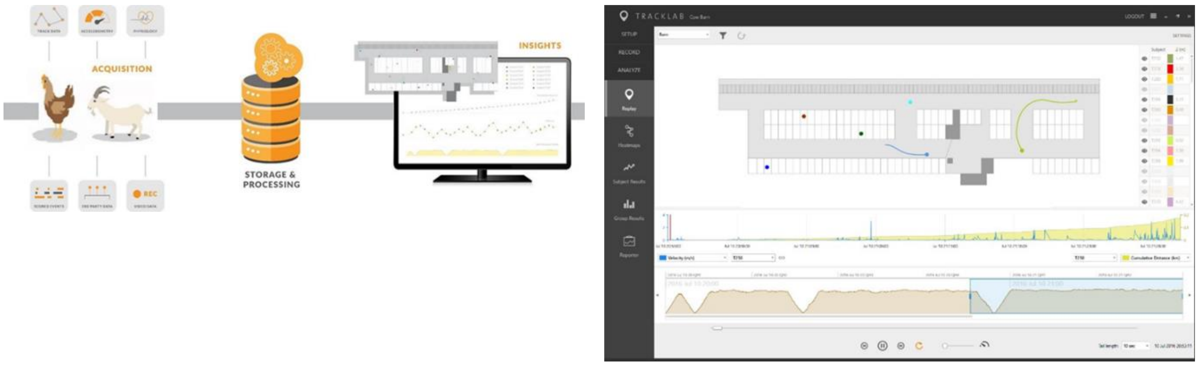

Figure 3-7. Schematic view of the TrackLab tool (left panel); Animal location replay on barn floorplan in TrackLab (right panel).

TrackLab allows to import location data collected in real-time. Tracks can be visualized on a map within the software on bitmaps, such as a floor plan of a particular barn in which the study is conducted. The software allows to visualize tracks of multiple objects simultaneously, see Fig. 3-7.

The analysis functionality of TrackLab provides a range of statistics relevant for analysis of location and movement, including various speed and distance variables. Statistics can be calculated for complete tracks or for specific zones.

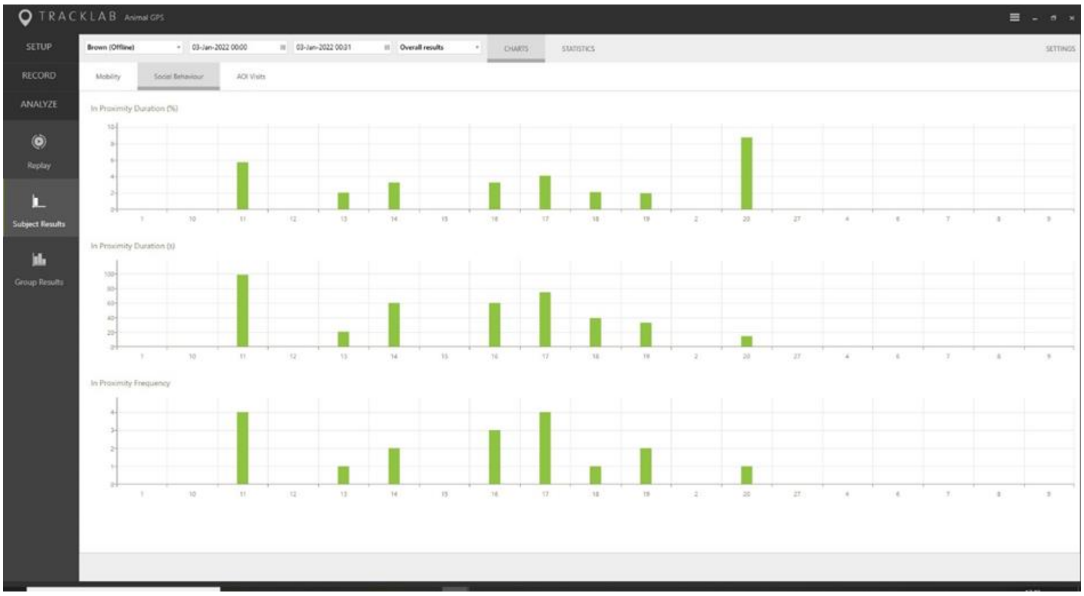

Figure 3-8 Animal proximity analysis in TrackLab.

The location of animals forms a strong indicator of their actions, both for motor behaviour such as walking and lying, as well as eating and social or aggressive behaviour (for instance feather pecking and piling behaviour of chicken). Behaviour classification models are developed based on the large-scale data collection using minimally invasive methods, i.e. tag

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°731014

attached to the collar of an animal or as backpack on smaller animals (like chicken or ducks), see Fig 3-9.

Figure 3-9 Backpack for chicken to mount UWB tag (left) and collar with UWB tag adapter for larger animals (like cow or sheep)

The animal facility where the experiment is conducted should be equipped with IP65 anchors (sensors) to process the UWB signal and transfer any sensor data (Fig. 3-10). This is compatible with the biosafety conditions required.

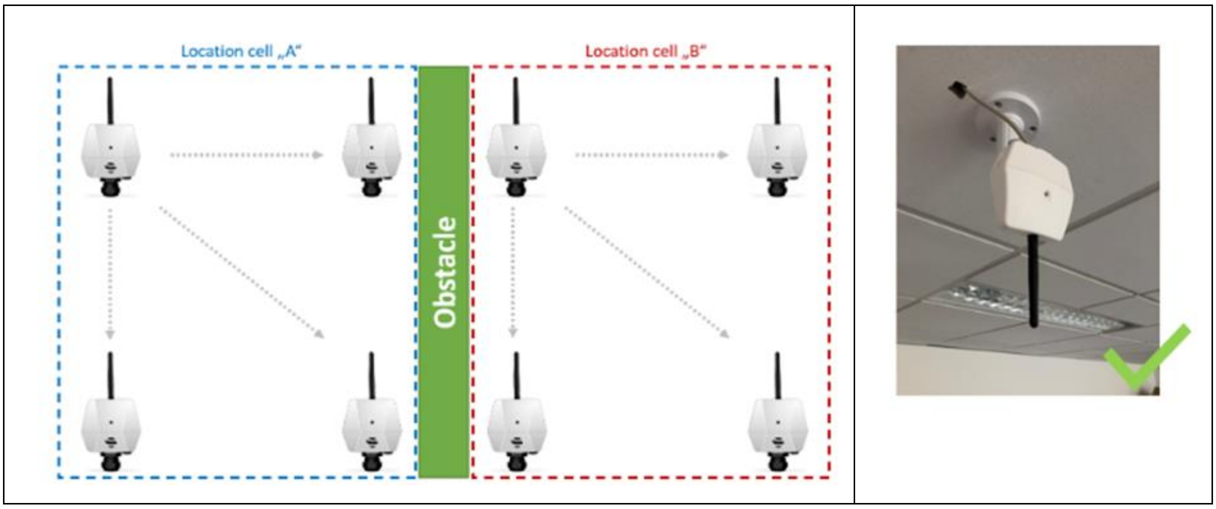

Figure 3-10 UWB system: Anchor deployment in a BSL facility.

The features of the TrackLab tool are listed below:

Automated data collection, with options for continuous and scheduled tracking - Track and analyze up to 100 subjects simultaneously; follow subjects with an accuracy of up to 10-20 cm (depending on the environment); process and analyze your data real-time with a maximum sampling rate of 20 Hz; real-time data collection & data processing and visualization; insights into animal activity, place preference, social behaviour, feeding and drinking behaviour, animal movement, etc.; data visualization with graphs and diagrams. Results are presented per individual animal, experimental group, region, and time interval

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement N°731014

and the export of data and results to csv files which can be imported in statistical programs like SPSS and The Observer XT.

System was used by VetBioNet partners: Noldus, WBVR, INRA, APHA, ANSES, TPI

3.1.1.6 ArUco marker tracking

One of the drawbacks of a UWB tag as described in previous paragraph is the weight of the tag and the options to attach a tag to an animal. This is especially true for small birds, like chicken or ducks where the weight of the tag with casing (in total 36 gram) is too heavy. The same applies to piglets and pigs where it is really a challenge to attach a tag to the animal, especially in a group housed setting which is normally the case. An alternative of the investigated techniques is the use of a small light-weight marker on the animal.
A known technique for computer vision applications to estimate the position of objects is to use binary square fiducial markers. An ArUco marker is a synthetic square marker composed of a wide black border and an inner binary matrix which determines its identifier (id). The black border facilitates its fast detection in the image and the binary codification allows its identification and the application of error detection and correction techniques.

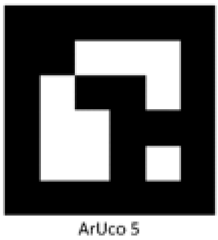	ArUco markers are: Much simpler than QR codes; Orientation invariant; Light and small tag is possible; Not expensive to create; Scalable

Figure 3-11 ArUco marker and relevant characteristics

ArUco markers are attached to a lightweight harnass for chickens to track individual movement (Fig 3-12).

Figure 3-12 ArUco markers attached on backpack on chicken

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°731014

A prototype ArUco marker detection software was developed using computer vision algorithms to detect and identify the location of the markers in video recordings (MP4 files). Video recordings are postprocessed to generate csv formatted text files with timeseries of location data from the marked animals. These text files can be imported in the TrackLab software for visualization and analysis of single or grouped animals.

Figure 3-13 Analysis of movement behaviour of chicken with ArUco markers in TrackLab

System was used by VetBioNet partners: Noldus, ANSES, WBVR

3.1.2 Tools to measure physiology

3.1.2.1 Overview of used technologies

In order to get more insights into the internal effects of treatment a variety of animals was equipped with a device to measure changes in physiology, with a primary focus on body temperature. Choice for a specific device depended on the species, environment and overall study design. Within VetBioNet, a wide variety of solutions for the measurement of body temperature were compared in order to come to the best solutions for each context. The chosen solutions can be found in table 3-1.

Main criteria for choosing a specific solution were: the range on which the sensors could function, either through real-time streaming of data or uploading logged data, the number of animals that simultaneously could be assessed and the amount and duration of data that could be collected. Most devices were implanted (see table 3-1), as this appeared to be the most reliable placement for accurate data collection. Devices were either implant-based (TSE & Emka), with leads that needed to be attached internally for the measurement of biopotentials, or capsule-based (BodyCap and Star-Oddi), which could be both ingested and implanted. The implant-based solutions had the advantage of being able to measure a variety of physiological signals (next to body temperature), with the cost of a more complex placement within the organism. Data from each specific solution was collected using the accompanying software.

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement N°731014

These packages not only acquire physiological data, but also pair datapoints with a timestamp. In order to combine physiological measurements with behavioural data, data had to be synchronized. Where behaviour was analyzed based on manual annotation of video data, MediaRecorder or Viso (both supplied by Noldus Information Technology) was used to collect

Table 3-1 *Overview of choses solutions for physiological measurements.*

Species	Manufacturer & device	Application method	Size	Range	Battery life
Cattle	BodyCap (Anipill)	Ingested (with weight), s.c. implanted	17.7mm x 8.9mm	1-5m	1-10 months
Sheep	BodyCap (Anipill)	Ingested, s.c. implanted	17.7mm x 8.9mm	1-5m	1-10 months
Alpaca	BodyCap (Anipill)	Injected, s.s. implanted	17.7mm x 8.9mm	1-5m	1-10 months
Pig	BodyCap (Anipill)	s.c./i.p. Implanted	17.7mm x 8.9mm	1-5m	1-10 months
Chicken	BodyCap (Anipill)	Ingested	17.7mm x 8.9mm	1-5m	1-10 months
Rat	TSE Systems (Stellar TA-XS)	Implanted	20mm x 15mm	3-5m	0.4-2.5 months
Hamster	Star-Oddi (DST nano-T)	s.c./i.p.Implanted	6mm x 17.5mm	20cm	≤28 months
Hamster	BodyCap (Anipill)	s.c./i.p.Implanted	17.7mm x 8.9mm	1-5m	1-10 months
Fox	Emka Technologies (TEL+M1_TA)	Implanted	33mm x 19mm	3-5m	≤85 days
Ferret	Star-Oddi (DST micro-T)	i.p. Implanted	8.3mm x 25.4mm	20cm	≤21 months
Ferret	BodyCap (Anipill)	i.p. Implanted	17.7mm x 8.9mm	1-5m	1-10 months

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°731014

video material. Using this software package, timestamps are automatically added to the video for synchronization purposes. Physiological data and video material were imported into The Observer XT (supplied by Noldus Information Technology), which automatically synchronized the data on the basis of supplied timestamps. In The Observer XT, behaviour could be annotated and used for further analysis. For those species from which behaviour was assessed using specific sensors (e.g. cattle), behavioural and physiological data were combined in TrackLab (supplied by Noldus Information Technology).

3.1.2.2 Emka Technologies

An implant-based device, TEL+M1_TA, developed by Emka Technologies was used in to monitor foxes. The device has to be implanted intraperitoneally and is suitable for animals above 200 grams. Next to body temperature, the device can measure ECH, blood pressure and activity. Per four devices, one receiver is required, with a maximum of 32 animals using eight receivers per system. Data transmission to the receivers can take place from a maximum distance of 3-5 meters.

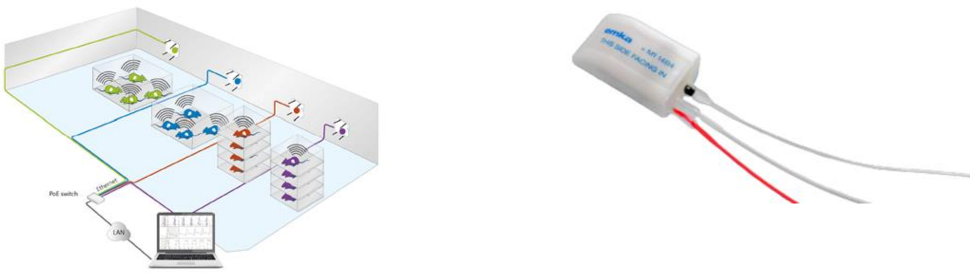

Figure 3-14 Figure 18. Schematic drawning of a setup making use of the Emka Technologies TEL+M1_TA; probe

System was used by VetBioNet partners: ANSES

3.1.2.3 BodyCap

A capsule–based device, Anipill, developed by BodyCap was used in combination with ungulates, chicken, hamsters and ferrets. The device can be injected, ingested or implanted in the peritoneal cavity in animals above 200 grams and only measures body temperature. Per eight devices, one receiver is required. Data transmission to the receivers can take place from a maximum distance of 1-5 meters, depending on the animal species, weight and environment.

System was used by VetBioNet partners: WBVR, FLI, INRAe, ANSES

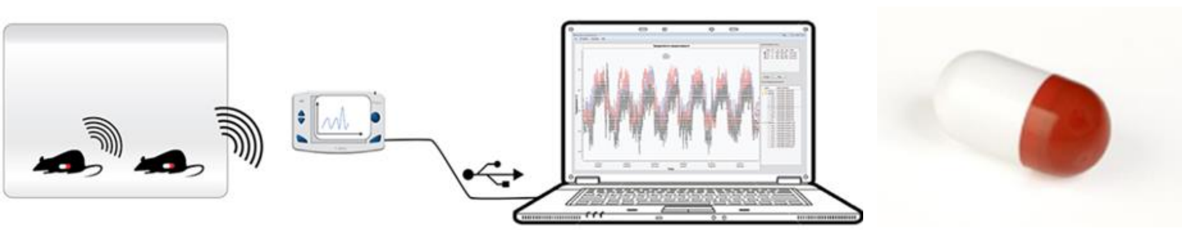

Figure 3-15 Schematic drawing of a setup making use of the BodyCap Anipill; capsule right

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°731014

3.1.2.4 Star-Oddi

From Star-Oddi the DST micro-T was implanted in ferrets and the DST nano-T was used in combination with hamsters. These devices can be ingested or placed intramuscular or intravaginal. The size and weight of the species determined which Star-Oddi device was used, as the DST nanoF-T could be implanted in smaller animals while the DST micro-T is suitable for larger animals. Next to body temperature, heart rate could be measured. Per 10 devices used, one receiver is required, which can be at a distance of a maximum of 20 centimeters from the devices.

System was used by VetBioNet partners: WBVR, FLI, ANSES

3.1.2.5 TSE Systems

For use in rats, TSE Systems provided the Stellar TA-XS, which had to be implanted either intraperitoneally or subcutaneously. The TA-XS was suitable for animals starting at 100 grams, although TSE Systems supplies comparable devices for a variety of animals. In addition to body temperature, these devices can measure ECG, blood pressure, EEG and activity at a distance of 3-5 meters from the receiver. Up to eight devices can transfer data to a single receiver.

System was used by VetBioNet partners: FLI

3.2 User experiences with various technological approaches to study behavioural or physiological changes in infection studies

The monitoring of behaviour in infection studies is focussed on the identification of changes in activity patterns due to the development of disease and aims to detect onset of disease or disease deterioration as early as possible. In contrast to short term observations with often defined behavioural provocations of individually kept animals in for example stress studies, infection studies are needing observations over longer periods (24/7) for several days/weeks. The various technological approaches as described above (3.1) have been applied in carnivores (foxes, ferrets, cats), pigs, ruminants (cattle, sheep), poultry (chicken, ducks) and fish (sea bass) studies. Experiences of users are compiled in appendix tables 5.1 – 5.5. Users appreciate the additional information for the interpretation of clinical disease development. For example, a high accordance was seen between activity changes and development of fever in various species and also interestingly behaviour changes were identified prior those observed by biotechnician/care takers. The added value of behavioural observations was broadly appreciated and also considered to be valuable for improved detection of human end points.

However, technical advances still need to be made to be able to use behaviour monitoring routinely in infection studies. Researchers struggled with various short-comings of the available technique and several bottlenecks have been identified. Infection studies with farmed and laboratory animals are performed in group of animals and usually in a confined area of a cage or an animal room. The behaviour of the group can be informative and compared between treatment groups; during the project time various studies have analyzed

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°731014

group behaviour. In pigs it was studied after infection with a high pathogenic PRRSV infection by analysing video recordings with the program EthoVision and changes have been demonstrated earlier than recording of clinical signs and at a similar time point as the development of fever (Fig.13-16). In cats group behaviour was used to compare effects of a SARS-CoV-2 infection, where clearly could be shown that no differences were observed between infected and uninfected groups. In fish studies group behaviour has been analysed after viral infection but, in infection studies it is preferred to follow individuals instead of groups. Also of interest is to distinguish individual activities like movements, locomotion, eating, drinking or social interaction with a high spatiotemporal resolution. Identification of individuals of alike-looking animals make visual distinction difficult, if possible at all. None of the current tagging systems, i.e. colour tags, are fully supporting these requirements. Promising attempts were made by using ArUco markers in combination with video-assisted analysis, however, dirt or feathers on the markers during a study interrupted continuous recordings. Ultra-wide-band tracking offers the chance to follow the locomotion of individuals in a group. However, small distances in and furnishing of animal rooms, especially metal structures limit the use of UWB tracking as seen in studies with pigs and lambs. A further disadvantage for this system is the necessity to equip rooms in a laborious way with receivers, which are de-installed after a study and need to be re-installed for use in a following study. In one pig study it appeared that movement behaviour can be measured in one animal room and distinguish between individuals, but comparisons with recordings from a second room appeared difficult because the results were very different without evident differences in other clinical findings. Especially in infection studies consistent recordings between rooms are necessary as the risk of cross-infections requires treatment groups to be housed in different animal rooms of similar design. The use of UWB tracking is also limited by the weight of the device, which makes it only usable in larger animals. Other accelerometer systems to measure activity appear to be a feasible option to record changes in X-Y-Z axis in time in various species, but it needs additional information or algorithms to extrapolate movements in locomotion or other activities. The advantage is that the weight of the accelerometers makes it possible to use it in a large range of species and species size. It will take additional time and studies to develop algorithms and make them useful in a general way. All behaviour recording and analyzing systems appeared to have huge data space requirements and although already software is developed for a number of these applications it was stated by a number of users that experienced data analysts are necessary to obtain a meaningful data extraction. The practical experience also taught that the current systems are useful and appreciated in single, dedicated studies, but not yet so far advanced, that they can be used as a regular practice in the current facilities. Data analysis is also relying on logged data and online interpretation of data, which would be required for the use in determining humane endpoints is not yet achieved.

The measurement of body temperature is a common practice and for a number of infectious diseases changes of body temperature or development of fever are highly informative about onset and course of the disease. Continuous logged or online available body temperature data are preferred above sporadic, manual data recording. The three different sensor systems (Star-Oddi, Anipill, Emka) need to be placed by invasive, surgical procedures. Users considered this as a disadvantage, but no significant side effects were reported and the obtained data were highly appreciated. Studies in pigs, but also in other species have shown that data can be reliably obtained from subcutaneous and intraperitoneal located sensors. As infection studies are regularly performed in a larger number of animals in a group, the

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement N°731014

expenses for these systems also determine the regular use of such systems. It appeared that high priced products are highly reliable, but that lower priced products are less reliable, but still capable to provide useful data. The use of infrared cameras to measure body temperature by skin temperature would be a non-invasive alternative; this was tried in small scale studies and it appeared to be able to identify fever in the study with wild boars, but correlation with rectal temperature is not consistent and the system was inferior to the sensor systems as shown in a cattle study.

Figure 3-16 Group behaviour of pigs after PRRSV 2 infection addressed by video analyses with EthoVision.

4 Conclusions

In the project period a lot of experience has been obtained to measure activity and body temperature over a long period of time with various systems. The simple translation of laboratory animal systems to infection studies in high containment conditions with a high diversity of animal species appeared to be challenging. Technical drawbacks like the interference with cage and pen furnishing, especially when containing metal materials or the life time of battery power or reflection of light through glass walls requested to find solutions for each individual study. Visual recognition of individual animals appeared still to be difficult for animal used in the studies and various tagging systems have been applied, but further development is needed to be able to be used routinely. An underestimated issue is the enormous amount of data, which is produced due to the continuous measurements and the complex data analysis. Although algorithms and software for a number of analyses are available, it became evident, that specialized personnel is necessary to extract and interpret the data.

Overall, the added value of continuous data recording of either activity or body temperature for the interpretation of infection studies has been underlined by the results of studies in various species during the project period. The experienced problems are highly useful for

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°731014

further advancements of different technologies. Although a validation of a specific system was not been achieved, the obtained experiences across different institutes and diverse species have resulted in increased interest and efforts to achieve improved systems.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°731014

5 Annexes

Table 5-1 Experiences and conclusions from various studies in carnivores with telemetric devices and behavioural monitoring

Species/pathogen	Housing (single/groups)	Technique	Hardware / software details	Supplier of tools	Aim of project [VetBioNet partners involved]	Results	Con's	Pro's
Fox/Rabies	Single	Implanted telemetry	Body Temperature (every 5 min) ECG [Emka]	Emka Technology	Study early symptoms and behaviour of four foxes under experimental infection with rabies virus to identify, as early as possible, end-points in the course of the infection. [ANSES-Nancy, Noldus]	The implant data demonstrated hyperthermia and hypothermia compatible with the development of the infection and not measured in standard protocols (rectal temperature is not measured in rabies foxes).	Quite large size (suitable for fox). Battery for 2-3 months. Positionning requires surgical intervention (specialised skills). High cost of individual implants, cost of receiver.	Data transfered live to computer with Emka software, with high positive value for detecting end-points (major objective of this project). Very well tolerated after IM implantation and SC probes. Excellent technical follow-up, for preparing the project, and analysing the data.
		Video annotation	The Observer [Noldus]	Noldus Information Technology		Preliminary qualitative video analysis revealed that stage 3 specific symptoms were detectable on video much earlier than by direct observations traditionally used for identifying end-points.	Manual entry of data in the software The Observer. High cost.	Specialised for analysing behaviours and symptoms. Link with implants data. In future experimental studies, telemetry (videos and if possible implants transmitting real-time data) will be additionally used to refine time-points and reduce animal suffering.
Ferret/Influenza H7N9	group	Data logger (intra abdominal)	Body Temperature (DST micro-T) [Star-Oddi]	Star-Oddi	Vaccine efficacy trial. Monitoring temperature increase as adverse event to vaccination & development of fever in response to H7N9 inoculation. [WBVR]	Body temperature differences after challenge between treatment goups correspond well with other clinical, virological and pathological findings.	Needs surgical, invasive procedure which requires vet expertise; probes are very costly; data are logged on transponder, i.e. only retrospective results.	Logged temprature data with very reliable data logging, easy to extract and data.
Ferret/Influenza H1N1	group	RFID transponder (intra abdominal)	Body Temperature [Bodycap; Anipill]	Bodycap	Vaccine efficacy trial. Monitoring body temperature increase in response to vaccinatiomn or H1N1 inoculation. [WBVR]	Body temperature differences after challenge between treatment goups correspond well with other clinical, virological and pathological findings.	Needs surgical, invasive procedure which requires vet expertise. Transmission coverage not always 100% resulting in missing data. Battery life limited (dependent on sample frequency).	Real-time transmission of data (RFID); less costly than other systems.
Cat/SARS-CoV-2	group	Video tracking	EthoVision [Noldus]	Noldus Information Technology	Pathogenesis trial. Detecting a possible (subclinical) reduction in activity in response to SARS-CoV-2 inoculation by continuous monitoring. Analysing pixel changes. Compare and align results to daily clinical observations. [WBVR]	After SARS-CoV-2 infection, observation are in line with absence of clinical symptoms. Be aware not to include recording periods with human interactions / animal handling. Camera position & room design should be considered carefully at beforehand.	Pixel change analysis only provides group-level activity. Data need to be extrapolated if animals are lost during monitoring. Data were only suitable for retrospective analysis.	Gives an impression on impact of infection on behaviour on group level.

Table 5-2 Experiences and conclusions from various studies in pigs with telemetric devices and behavioural monitoring (1)

Species/pathogen	Housing (single/groups)	Technique	Hardware / software details	Supplier of tools	Aim of project [VetBioNet partners involved]	Results	Con's	Pro's
Pig/PRRSV2	group	Video tracking	EthoVision [Noldus]	Noldus Information Technology	Determination of virulence of high pathogenic PRRSV2 strain. [Noldus, WBVR]	Decrease in activity post PRRSV challenge was observed 24 hours earlier than with clinical score (& feed intake) and simultaneous with increase in rectal temperature.	Pixel change analysis only provides group-level activity. Data need to be extrapolated if animals are lost during monitoring. Only group analysis possible yet.	Detection of early clincal disease signs on group level;
Pig/APP	group	RFID transponder (intra abdominal & subcutaneous)	Body Temperature [Bodycap; Anipill]	Bodycap	Monitoring fever after *A. pleuropneumoniae* inoculation by continous temperature measurements compared to 1x daily rectal temperature measurements. Compare results from various sites of transponder location, i.e. subcutaneous with intraperiotoneal location. [WBVR]	Data from both s.c. locations similar to i.p. location and rectal measurements.	Surgical procedures required; transmission coverage not always 100%. Battery life limited (dependent on sample frequency).	Subcutaneous application is minimally invasive and reducing stress by animal handling.
		Video tracking	EthoVision [Noldus]	Noldus Information Technology	Monitoring activity in response to APP inoculation by analysing pixel changes. Compare and align results to (currently in-use) daily clinical observations and temperature measurements. [WBVR]	Changes on group basis were insufficiently useful; therefore individual tacking was tried, but was not successful.	Pixel change analysis only provides group-level activity. Data needs to be compensated if animals are lost during monitoring.	n/a
Pig/HEV3	group	Ultra wideband tracking	TrackLab [Noldus]	Noldus Information Technology	Development of a HEV challenge model in pigs. Detecting activity changes in response to HEV inoculation by continous monitoring. Compare and align results to daily clinical observations. [WBVR]	No thorough analysis due to incomplete & inconsistent dataset. Within animals, no change in activity (pattern) after HEV infection was observed (measured as distance moved). This is in line with clinical observations.	Laborious with regard to installation / calibration (accuracy 10-30 cm). Not fit for smaller animal rooms (loss of signals at the edges); equipment relatively vulnerable to disorders, needs regular check by technicians (we experienced a broken anchor and dead batteries were noticed late); variable measurement frequency (due to sleep mode); limited information about type of behaviour. Activity that does not result in spatial displacement or movement in Z axis is not detected. Requires informatics skills and time.	Analysis of activity on individual level (incl. interactions and location).

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°731014

Table 5-3 Experiences and conclusions from various studies in pigs with telemetric devices and behavioural monitoring (2)

Species/pathogen	Housing (single/groups)	Technique	Hardware / software details	Supplier of tools	Aim of project [VetBioNet partners involved]	Results	Con's	Pro's
Pig/ASF	BSL3	IR thermography; comparison with rectal temperature	FLIR T450sc	FLIR	Animals were culled throughout the incubation period and only 4 boars were left to develop full clinical disease; daily (a.m.) checks starting 3 days prior to experimental infection until 6 days post infection (study end). [APHA]	IR images were extracted from video clips because still pictures are less suitable for moving animals. Two hairless areas of interest were identified for temperature analysis: the area next to the eye and the eye itself. In general, eye temperature was considerably lower than skin temperature (could be more than 10 degrees C) and skin temperature was lower than rectal temperature (approximately 3 degrees C). There was a fairly good correlation between rectal temperature rises and skin or eye temperature rises, particularly in febrile animals, but eye temperature was inferior, possibly due to a smaller area for measurement in combination with eyelashes partly concealing the cornea and possibly leading to erroneous measurements. The findings of this pilot study suggest that skin temperature could be used to detect fever in wild boar, at least under controlled environmental conditions.	There was a fairly good correlation between rectal temperature rises and skin or eye temperature rises, particularly in febrile animals, but eye temperature was inferior.	The findings of this pilot study suggest that skin temperature could be used to detect fever in wild boar, at least under controlled environmental conditions.

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement N°731014

Table 5-4 Experiences and conclusions from various studies in ruminants with telemetric devices and behavioural monitoring

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°731014

Species/pathogen	Housing (single/groups)	Technique	Hardware / software details	Supplier of tools	Aim of project [VetBioNet partners involved]	Results	Con's	Pro's
Sheep/RVFV	group	Ultra wideband tracking	TrackLab [Noldus]	Noldus Information Technology	Vaccine efficacy trial; activity levels in response to RVFV inoculation, comparing vaccinated and non-vaccinated sheep by continous measurements. [Noldus, WBVR]	After RVFV infection, a difference in activity (distance moved) between vaccinated and non-vaccinated animals was observed (in line with clinical symptoms).	Laborious with regard to installation / calibration (accuracy 10-30 cm). Usefulness in smaller animal rooms (18m2) limited; limited information about type of behaviour. Activity is exclusively related to spatial movement (20 - 30 cm) and not detected in Z coordinate.	Analysis on individual level (activity, interaction, location)
sheep/Toxo-plasma	group	Video tracking	ArUco marker	Noldus Information Technology	To develop a method for tracking changes in individual activity (and specific behaviour) of sheep over time by use of video monitoring and accelerometers to support decision making with regard to humane endpoints. Compare two locations of accelerometer (ear & back). [Noldus, WBVR]	Activity index (simple proxy of activity based on standard deviation of signal in x, y and z axes) was calculated and revealed a clear circadian rhythm. Activity during day dropped at day ~4 after infection in line with increase in body temperature. Activity during night increased at day ~4 after infection, indication of restlessness? We found comparable data for both locations (ear & back).	Individual tracking possible, but needs a lot of preparatory work in animal room.	Indivual tracking incl. eating and drinking behaviour
		accelerometers	MOX1 [Maastricht Instruments]	Maastricht Instruments			Need of data scientist to process / evaluate the data and to calculate activity index. Classification of patterns & annotation of behaviour based on accelerometer data is not simple and cannot be applied generic (requires case-based manual training of the model with use of corresponding video images).	Activity analysis on. individual level
Cattle/Foot and mouth disease	group	Ultra wideband tracking / Video annotation	TrackLab [Noldus] / Viso [Noldus]	Noldus Information Technology	Measuring automatic behavior and activity in cattle challenged with Foot and Mouth Disease Virus. [Noldus, TPI]	First testing of the automatic behavior recognition model. Could retrieve the first behaviors such as walking, standing still, laying, feeding, and drinking. First recorded spatial data needs to be optimized for further conclusions.	Too much movement noise in the data due to high recorded date for accelerometer recordings. The room was small which prevented clear spatial movement measurements of the rather large animals.	
Alpaca / Influenza C&D	group	Temperature capsule (ingested and subcutaneous)	Body Temperature [Anipill]	BodyCap	To establish a clinical scoring system for influenza C and D virus infection in alpacas, which included body temperature. [APHA]	Temperature correlation with rectal temperature (gold standard) dependent on the location of Anipill; e.g. closer to rectal temperature if in the stomach compared to subcutaneous location.	Range of the monitor was a challenge in larger animals due to the blocking of the signal. The monitor needed a constant power source.	More objective and continuous temperature results without stessing the animals.
Cattle/Mycobacterium bovis	BSL3 or none (controls)	IR thermography, comparison of rectal temperature with Anipill; measurements during skin testing (SICCT - single intradermal comperative cervical tuberculin test; intradermal injection of avian and bovine tuberculin, measurements at regular intervals until 72 hours post injection); comparison with skin test result and postmortem findings (e.g. visible or no visible lesions)	FLIR T450sc [FLIR], Anipill [Bodycap]	FLIR, Bodycap	Body temperature measurement compared by IR camera and Anipill. [APHA]	Anipill is more reliable in detecting changes in core body temperature post-SICCT than rectal temperature as measurements are continuously but connectivity of Anipill continues to be an issue as it is not designed for larger species so data needs to be retrieved from the Anipill post mortem. IR analysis ongoing but not useful to distinguish animals based on skin temperature difference amplitude alone; probably needs to include a time element.	IR analysis not useful to distinguish animals based on skin temperature difference amplitude alone; probably needs to include a time element.	Anipill is more reliable in detecting changes in core body temperature post-SICCT than rectal temperature as measurements are continuously but connectivity of anipill continues to be an issue as it is not designed for larger species so data needs to be retrieved from the anipill post mortem.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°731014

Table 5-5 Experiences and conclusions from various studies in poultry with telemetric devices and behavioural monitoring

Species/pathogen	Housing (single/groups)	Technique	Hardware / software details	Supplier of tools	Aim of project [VetBioNet partners involved]	Results	Con's	Pro's
Ducks/No pathogen healthy animals)	6 x 2 groups (one with harness, one without)	Video tracking	ArUco marker	Noldus Information Technology	Tracking individual ducks in groups [Noldus, ANSES]	Detection software on basis of computer vision developed	ArUco marker not always detectable (dirt, feathers),i.e. missing data; light sensitive;	Individual tracking achievable, but not yet fully functional.
chicken/Avian Influenza	group	Video tracking	ArUco marker	Noldus Information Technology	To develop a method for individual activity tracking of birds by video monitoring and if possible develop automatized identification of endpoints. [Noldus, WBVR]	Obtained dataset: only 50% detection rate, including longer gaps in the data > activity levels were not analysed	Proper attachment on the (growing) animal is challenging. Visibility within the group is poor, also due to feathers and filth on the codes. Blurred images during movement.	Individual tracking achievable, but not yet functional.
Chicken/ IBDV	group	Ingested capsule	Body Temperature [Anipill]	BodyCap	The objectives of the experiments were first to test the safety of the system and then to evaluate its interest in animal infectiology research using inoculation by a very virulent chicken virus. [APHA]	Oral administration of the capsule was easily performed for all animals. After introduction into the esophagus, soft massaging of the throat ensured movement of the capsule down the crop. At the end of the experimental period, that is 14 days after implantation, during necropsy, all capsules were found in the gizzard, and no macroscopic gizzard lesion was found in implanted nor control animals.	Even if cloacal and capsule temperatures were correlated, it was found that cloacal temperature values were consistently higher than those recorded by the capsules.	More objective and continuous temperature results without stessing the animal.
chicken/AIV	BSL3	IR thermography	FLIR T450sc	FLIR	Body temperature during AIV infection; pictures/ videos taken when animals were held, images acquired pre-challenge and then daily until end-point (14 d post challenge); three survivors. [APHA]	IR image analysis better from videos (three frames analysed). Only hairless areas useful (cutaneous appendices); eye temperature differed significantly between left and right side (due to independent or intentional pupil size constriction?), which makes it less useful. Ear lobe temperature assessment most promising; temperature or amplitude higher in non-survivors. Head temperature assessment (maximum) may be sufficient as it covers all cutaneous appendices; eye temperature generally lower than skin temperature.	Legs not included as view obstructed by handler. Comb temperature less useful as comb grew over time and in some instances folded over so temperature data difficult to extract.	Head temperature assessment (maximum) may be sufficient as it covers all cutaneous appendices; eye temperature generally lower than skin temperature.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°731014

Table 5-6 Experiences and conclusions from various studies in fish species with telemetric devices and behavioural monitoring

Species/pathogen	Housing (single/groups)	Technique	Hardware / software details	Supplier of tools	Aim of project [VetBioNet partners involved]	Results	Con's	Pro's
Fish/	both (tagged or not)	Video tracking	EthoVision [Noldus]	Noldus Information Technology	The aim of the project was to develop a method for individual video monitoring of rainbow trout and automatized identification of endpoints in infectiology experiments. In the experimental protocols for infectious diseases research, refinement is difficult to implement because of the rapidity of the disease outcome and the large number of fish per batch, a necessity to conduct statistical analyses and to ensure a relevant biological interpretation. The determination of the end point leading to the exit of the animal from the protocol, rely on scoring established through daily monitoring of phenotypic features (loss of reactivity, changment of feeding habits) observed by the experimenters. Tracking of individual swimming trout needs the proper detection of the fish position on each frame of a movie acquired with video camera providing continuous monitoring of fish behavior. [INRA-IERP]	Colored tags sewn to the dorsal fin allow the selection of individual fishes in the tank. These optical objects are conjugated to the detectors and tracked afterwards. 7 different colors were selected based on the ability to detect and differentiate them with the camera of the set up. Two tank chambers were recorded simultaneously (infected and naïve conditions) during the whole experiment, Observations were carried out in a two chambers set up containing home made tanks with transparent Plexiglas walls and a white bottom. Behavior of infected fish was assessed by measuring the distance, the maximal velocity (swim burst), the position to the wall or the length of the stationary phases. Overall, these parameters were defined as independent values for each fish in each condition up to the death of the animal.	Detecting problems and locating errors due to i) total internal reflection on the glass/air interface at the origin of a second image of the fish (ii) reflections at the water/air interface which can dazzle the upper camera, (iii) condensation on the glass/air interface can impair the observation by hiding the fishes, (iv) bubbling of the water oxygenation into the tank adds on the one hand moving objects that can puzzle the detection algorithm and on the other hand wavelets at the surface. Recommendation: To implement video monitoring with a 2nd camera from a side view in addition to the one from the top. This will be used to track the animals in the whole volume of the tank and extract xyz coordinates. Infected animals used to present typical alterations of feeding habits evolving in freezing behavior that will be trace in 3D by a two camera setting. Integration of objectively characterized parameters recorded continuously from the infection start until the death of the fish: development of biostatistics methods, data integration, artificial neural network. This will be instrumental to build predictive systems and establish a referencing system to diagnose disease development.	n/aLucas Noldus: No Pro's at all? I thought this study was successful. The video tracking of the colored beads worked well, so individual fish tracking was possible.
Fish/Betanodavirus (NNV)	groups	Video tracking	EthoVision [Noldus]	Noldus Information Technology	Behaviour of infected sea bass versus non-infected was observed. Sea bass were infected with Betanodavirus (NNV) by intraperitoneal injection while the contrl group was mock infected with PBS. [IZSVe]	VNN infected fish reduced velocity, distance moved and schooling compared to the mock infected group. Shoaling was the easier parameter to detect. Instead, abnormal swimming pattern (whirling) and floating belly up were the most difficult parameters to be identified by the software. Good correlation between clinical signs insurgence and viral agent detection was observed and the software allowed to identify earlier than usual the fish abnormal behaviours.	The main constraint was the impossibility to film continuously the fish due to the turbulence of the water surface caused by the air supply. In fact oxygen concentration in the water declined rapidly without air supply and after 30 minutes O2 reached critical level for fish.	This is promising since video tracking could be used for prompt action, treatment and humane end point determination.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°731014