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Pilot study of long term anaesthesia in broiler chickens.

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Abstract

Objective

To provide stable anaesthesia of long duration in broiler chickens in order to perform a terminal caecal ligated loop procedure.

Study Design

Prospective experimental study

Animals

Seven clinically healthy broiler chickens (Gallus domesticus) aged 27-36 days, weighing 884 to 2000 g.

Methods

Anaesthesia was induced and maintained with isoflurane in oxygen. All birds underwent intermittent positive pressure ventilation for the duration. $P_{\bar{E}}CO_{2,}$ SpO_2, heart rate and oesophageal temperature were monitored continuously. Intraosseous fluids, warming pad and intramuscular butorphanol at 2 mg kg^{-1} q 2h were provided. Euthanasia by parenteral pentobarbitone was performed at the end of procedure.

Results

Stable anaesthesia was maintained in four chickens for durations ranging from 435 to 510 minutes. Two birds died or were euthanised after 130 and 330 minutes due to surgical complications and another died from anaesthetic complication after 285 minutes.

Conclusion and clinical relevance

Minimal anaesthesia duration is recommended in avian patients as they are often ill when presented. Long-term, stable anaesthesia is possible in clinically healthy chickens provided complications such as hypothermia and hypoventilation are addressed, and vital signs are carefully monitored. There are no previous reports describing monitored, controlled anaesthesia of this duration in chickens.

Keywords

anaesthesia, avian, chicken, ligated-loop, coeliotomy

Introduction

Much is written in clinical texts and literature about recommendations for safe anaesthesia in the avian patient, however surprisingly little is published about maintenance of long term anaesthesia. Fedde (1978) has reviewed the use of many agents used in avian anaesthesia, including phenobarbitone which provided anaesthesia of 24 hour duration. However, few details are given regarding the stability or monitoring of anaesthesia in these reports.

We report the results of a pilot study where long term anaesthesia was required for a caecal ligated-loop experiment studying bacteriophage therapy for Campylobacter jejuni (Connerton et al. 2011). The procedure was based on that reported by Van Deun et al. (2008), but with the objective of longer term anaesthesia followed by euthanasia rather than recovery.

Materials and Methods

Animals

Seven male broiler (Ross 308) chickens were obtained as day olds from a commercial hatchery and reared in a biosecure environment until day of procedure, between days 27 and 36 in accordance with the Home Office code of practice for the housing and care of animals used in scientific procedures. Birds were group housed until 20 days of age and individually caged thereafter. Feed and water were available ad libitum with a 12 hour light/dark cycle. All birds were considered to be healthy at pre anaesthesia clinical examination.

This study was carried out in accordance with UK and EU legislation. All procedures were approved by the Local Ethics Committee of the University of Nottingham and performed under Home Office licence.

Anaesthetic protocol

Feed was withdrawn on the morning of the procedure, and the birds weighed and their crops palpated to confirm no ingesta were present.

Birds were restrained manually with a towel, using a mask attached to an Ayre's T-piece circuit anaesthesia was induced with a vapouriser set to deliver 5% isoflurane (IsoFlo, Abbott Laboratories) in oxygen. Once anaesthetised each bird was intubated with an uncuffed orotracheal tube (Portex, Smiths Medical, UK) with internal diameter 2.5-4 mm, depending on bird size. Anaesthesia was maintained with isoflurane in oxygen and intermittent positive pressure ventilation was performed using a pressure limited ventilator (SAV03, Vetronic Services, UK). The trigger point was set to achieve normal inspiratory depth and the expiratory time adjusted to maintain an end tidal CO_2 ($P_{\bar{E}}CO_2$) target of 35 to 45 mmHg (4.7-6.0 kPa). Depth of anaesthesia was assessed using cardiovascular parameters and reflexes, isoflurane vaporiser settings between 2.5% and 3% were used. The bird was placed in dorsal recumbency between warm water filled gloves on an electronic heat mat and foam wedge at an angle of approximately 10^o. Heart rate (HR) and haemoglobin saturation (SpO_2) were measured using the pulse oximeter probe (placed on the wingweb or between toes) on a VM 2500 veterinary CO_2/SpO_2 monitor (Viamed, UK). $P_{\bar{E}}CO_2$ and ventilation rate (fV) were measured on the same unit. A flexible thermometer probe attached to a monitoring console (Minimon 7138B, Kontron) was introduced oesophageally to approximately the level of the heart. Body temperature (Tp), SpO_2, $P_{\bar{E}}CO_2$, HR and f_V were monitored continuously and recorded every 15 minutes.

A 21g hypodermic needle was placed in the proximal tibiotarsus to deliver lactated ringers (Vetivex 11, Dechra, UK) solution at 10 mL kg^{-1} $hour^{-1}$.

Butorphanol (Torbugesic, Pfizer, UK) was administered intramuscularly, into the superficial pectoral or thigh, at a dosage of 1 mg kg^{-1} in the first bird and 2 mg kg^{-1} every 2 hours in the subsequent 6 birds.

Surgical procedure

A midline coeliotomy was performed with parasternal flap extension to allow exteriorization of intestines and caeca for ligation, sampling and injection. Further sampling was performed every 1-2 hours for a total of 6 hours. Between samplings the viscera were returned to the body cavity and the body wall temporarily apposed.

All birds were euthanised at the end of the procedure with overdose of parenteral pentobarbitone.

Results

Ages, weights and monitored parameters are shown in table 1. Birds 2,3,5 and 7 survived for the duration required for the experiment. Bird 1 died following a surgical complication during the coeliotomy, the technique was refined in subsequent surgeries. Bird 4 was euthanised after 330 minutes following detection of caecal thromboemboli.

Bird 6 died unexpectedly after 285 minutes of anaesthesia, this was noted as a sudden drop in $P_{\bar{E}}CO_2$ followed by loss of pulse oximeter trace and palpable heartbeat. No change in monitoring parameters were noted prior to this occurence.

Despite reducing airway pressure on entry of the body cavity and attempting to pack off with moistened swabs, at least one abdominal air sac was ruptured in all of the birds.

Airway pressure did not exceed 15 cm H_2O in any of the birds, once the body cavity was opened the pressure was reduced to as low as 4 cm H_2O to minimise volutrauma to the air sacs. The ventilation frequency rate was adjusted to maintain adequate ventilation as determined by $P_{\bar{E}}CO_2$.

The ET tube was replaced at least once per procedure as routine and changed immediately if any obstruction suspected. Thick mucus was often present after 2-3 hours of anaesthesia.

Discussion

A ligated loop study was selected for this experiment as it removes many variables encountered in alternative experimental designs. This approach should greatly reduce the number of animals required to obtain significant results as it removes inter-animal variation. This is in keeping with the principle of Replacement, Refinement and Reduction of animals in research (Russell & Burch 1959). The authors are unaware of any published reports describing a monitored, controlled anaesthesia of this duration. Several older texts describe ligated loop studies, but often the anaesthesia is not described in detail. We report these results to demonstrate that this model is viable so others may use it in future.

No blood haematological or biochemical testing was performed as these were all young, clinically healthy birds and results would have been unlikely to change the protocol.

Once anaesthesia was induced IPPV was initiated with no resistance or bucking of the ventilator. There was therefore no requirement for neuromuscular blockade. Butorphanol was included in the protocol as it has been demonstrated to have an isoflurane sparing effect in psittaciformes (Curro et al. 1994). The pharmacokinetics of butorphanol have recently been described in broilers by Singh et al. (2011), hence selecting the dose of 2 mg kg^{-1} every 2 hours.

Pulse oximetry for the estimation of haemoglobin oxygen saturation has been widely considered unreliable in avian species (Edling 2006). The equipment is calibrated for mammalian, not avian haemoglobin and tissues and tends to underestimate the haemoglobin saturation in birds (Schmitt et al. 1998). The Viamed pulse oximeter used in this study provided a very consistent trace and provided an audible alarm when the trace was lost. Validating SpO_2 data was not possible in this pilot study but could be performed in future studies. A pulse oximeter probe from the older Kontron monitor (Minimon 7138B) provided no trace or SpO_2 reading.

Bird 6, which died after 4.75 hours of anaesthesia was the heaviest and most muscled of the chickens anaesthetised. The “Sudden Death” syndrome (SDS) of broiler chickens tends to affect faster growing birds and although the aetiology is poorly defined it may be associated with cardiac arrhythmias (Crespo & Shivaprasad 2013). Given that this bird may have had the lowest cardiorespiratory reserve capacity within our cohort this is perhaps not a surprising occurrence. No gross lesions were apparent at post mortem, which can be consistent with SDS.

Clinical texts frequently emphasise the requirement for speed as avian patients requiring anaesthesia for procedures are rarely healthy (Edling 2006). Long-term, stable anaesthesia does appear to be possible in healthy chickens. As these were terminally anaesthetised for ethical reasons we have no data on recovery and survival after the procedures. The anticipated problems of hypothermia, hypoventilation and regurgitation were avoided or managed, and monitored parameters were within acceptable physiological limits. Studies such as Fedde et al. (1998) demonstrated the heart rate in conscious broiler chickens to be in the region of 360 beats minute $^{-1}$. Our data from anaesthetised birds in Table 1 will be of use for future studies.

The ideal feed withdrawal period for avian anaesthesia is conflicted by concerns of avoiding regurgitation, but maintaining adequate energy reserves for a very long procedure (Edling 2006). A small degree of regurgitation was noted mid-procedure in bird 6, which had the shortest feed withdrawal time of 2 hours, however the crop was palpably empty even when the feed was removed. The material was removed with cotton swabs, and was considered unrelated to the anaesthetic death as no material was noted in the trachea at post mortem examination.

As described elsewhere in the literature (Edling 2006), air sacs were ruptured during this procedure. Isoflurane pollution of the environment is inevitable, therefore adequate ventilation of the operative area is essential and charcoal filtered surgical masks should be considered.

Based on the results of this pilot study a larger scale experiment can be designed with further refinements including arterial blood gas analysis to validate the SpO_2 and $P_{\bar{E}}CO_2$ monitoring equipment. This will expand the data set of normal values for broiler chickens undergoing anaesthesia and supplement the findings of others that $P_{\bar{E}}CO_2$ correlates accurately with arterial concentrations (Edling 2006).

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Bird	Age (d)	Weight (g)	Duration (minutes)	SpO_2 (%)	HR (beats minute^{-1})	Ventilation rate (breaths minute^{-1})	$P_{E'}CO_2$ (mmHg) (kPa)	Oesophageal Temperature (^{O}C)
1	27	884	130	99 ± 1.04	251 ± 69.78	29 ± 7.49	44 ± 12.44 5.87 ± 1.66	38.8 ± 0.99
2	29	1100	480	99 ± 1.22	252 ± 37.70	16 ± 3.14	37 ± 12.73 4.93 ± 1.70	39.6 ± 0.76
3	30	1160	435	99 ± 0.00	284 ± 22.18	20 ± 4.21	47 ± 9.76 6.27 ± 1.30	39.6 ± 0.86
4	31	1140	330	99 ± 0.44	305 ± 29.70	19 ± 5.78	44 ± 11.02 5.87 ± 1.47	40 ± 0.95
5	34	1300	450	96.5 ± 4.15	293.5 ± 25.94	17.5 ± 4.52	44.5 ± 8.05 5.93 ± 1.07	40.7 ± 0.83
6	35	2000	285	99 ± 0.00	241 ± 44.27	19 ± 31.2	47 ± 12.85 6.27 ± 1.71	39.2 ± 1.23
7	36	1585	510	99 ± 0.00	271.5 ± 44.62	20 ± 5.12	38 ± 9.98 5.07 ± 1.33	40.55 ± 0.86

Table 1. Monitored variables in broiler chickens undergoing anaesthesia (median ± standard deviation)