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Untersuchungen zur Kolostrumversorgung von Kälbern im Bundesland Salzburg und Validierung eines Immunglobulin-Schnelltests

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1. Einleitung

1.1. Immunsystem des Kalbes

Die Plazenta des Rindes ist ein kodyledonärer synepitheliochorialer Plazentatyp. Dies hat zur Folge, dass keine maternalen Antikörper während der Trächtigkeit auf den Fetus übertragen werden können. Das bedeutet, dass Kälber ohne zirkulierende Immunglobuline geboren werden (Godden et al. 2019, Schnorr und Kressin 2001). Das Immunsystem der Kälber ist zum Zeitpunkt der Geburt zwar funktionsfähig, jedoch naiv (Weaver et al. 2000). Daher muss es unmittelbar nach der Geburt über die Fütterung von hochwertigem Kolostrum aufgebaut werden, um einem „Failure of Transfer of Passive Immunity“ (FTPI) vorzubeugen (Godden et al. 2019).

Über die Kolostrumaufnahme findet der „Transfer of Passive Immunity“ (TPI) von Immunglobulinen, vorrangig Immunglobulin G (IgG) (davon 85-90 % IgG1) statt (Godden et al. 2019, Smith et al. 1964). Es ist dies der Prozess der Übertragung von maternalen Immunglobulinen via Kolostrum zum Kalb. Der Erfolg dieses Prozesses hängt von mehreren Faktoren ab, wie etwa: Zeitpunkt der Kolostrumgabe, Qualität des Kolostrums, Keimgehalt im Kolostrum und die Fähigkeit des Kalbes, die Immunglobuline im Darm resorbieren zu können (apparent efficiency of immunoglobulin absorption) (Godden 2008, Godden et al. 2019).

Nicht nur Immunglobuline werden über das Kolostrum an das Kalb weitergegeben, sondern auch andere, sehr wichtige Substanzen, wie Wachstumsfaktoren, Hormone, antimikrobielle Faktoren, Leukozyten, Oligosaccharide, mRNA und Nährstoffe. Das genaue Zusammenspiel all dieser Faktoren ist aktuell noch nicht vollständig bekannt (Godden et al. 2019).

1.2. Einfluss von „Failure of Transfer of Passive Immunity“ auf die Gesundheit des Kalbes

Wenn die Versorgung des neugeborenen Kalbes mit Kolostrum nicht adäquat erfolgt, führt dies zu FTPI. Wie zahlreiche Studien belegen, sind die Konsequenzen, welche FTPI mit sich bringt, nicht nur aus ökonomischer Sicht, sondern auch bezüglich des Tierschutzaspektes, bedeutend (Mee 2013). Der Grund dafür ist, dass für Kälber ohne Immunglobuline die Morbidität und folglich auch die Mortalität steigen. Raboisson et al. (2016) konnten zeigen, dass im einem 95 % Konfidenzintervall die Konsequenzen von FTPI und das damit verbundene Risiko für die

Sterblichkeitsrate bei 2,12 (1,43-3,13), für Atemwegserkrankungen bei 1,75 (1,50-2,03), für Enteritis bei 1,51 (1,05-2,17) und für die generelle Erkrankungswahrscheinlichkeit bei 1,91 (1,63-2,24) lag. Eine andere Studie belegte, dass die Kälbersterblichkeit auf Betrieben, auf welchen mehr als 25 % der Kälber FTPI hatten, bei 5 % innerhalb der ersten zwei bis sechs Lebenstage lag (Tautenhahn et al. 2020). Aktuell beschäftigen sich sehr viele Studien mit dem Auftreten von FTPI und der Anzahl der betroffenen Kälber, wobei die Ergebnisse sehr stark variieren. So waren beispielsweise in einer schottischen Studie 14,1 % der 370 inkludierten Kälber von FTPI betroffen (Haggerty et al. 2021), in einer Studie aus den USA 19,2 % der inkludierten 2030 Kälber (Beam et al. 2009), 27,0 % der inkludierten 216 Kälber in einer Studie aus Deutschland (Sutter et al. 2020), 33,0 % in einer neuseeländischen Studie mit 3819 inkludierten Kälbern (Cuttance et al. 2017), 43,5 % in einer Studie aus der Schweiz mit 373 untersuchten Kälbern (Reschke et al. 2017) und 41,9 % in einer australischen Studie mit 253 Kälbern (Abuelo et al. 2019).

1.3. Kolostrum

1.3.1. Kolostrummanagement

Als qualitativ hochwertiges Kolostrum gilt jenes mit einer Immunglobulin-Konzentration von ≥ 50 g/l und einer gesamten Keimbelastung von < 100.000 colony forming units pro Milliliter (cfu/ml) und der Anzahl an coliformen Bakterien von < 10.000 cfu/ml (Buczinski und Vandeweerd 2016, Godden et al. 2019, McGuirk und Collins 2004). Hygiene bei der Kolostrumgewinnung und –verabreichung spielt eine essentielle Rolle, da Bakterien die Immunglobuline deaktivieren können, bevor sie vom Darm absorbiert werden können (Staley und Bush 1985). Des Weiteren sind Bakterien in der Lage, die Rezeptoren im Dünndarm, an welchen Immunglobuline binden sollen, zu blockieren, wodurch die Anzahl an absorbierten Immunglobulinen stark sinkt (Johnson et al. 2007). Den größten Teil der Immunglobuline machen IgG mit 87,3 % (davon ca. 85,0 % IgG_1) aus. Es kommen aber auch noch IgA (3,5 %) und IgM (9,2 %) vor (Kehoe et al. 2007). Da die Absorptionsfähigkeit von Immunglobulinen im Darm der neugeborenen Kälber innerhalb der ersten 24 Lebensstunden stark abnimmt und 24 bis 36 Stunden nach der Geburt nicht mehr gegeben ist (Stott et al. 1979), ist es essentiell, dass die Kälber so schnell als möglich mit hochwertigem Kolostrum versorgt werden. Ziel wäre, den Kälbern innerhalb der ersten zwei Lebensstunden mindestens vier Liter hochwertiges

Kolostrum (≥ 50 g/l IgG) zu füttern, um FTPI vorzubeugen (Buczinski und Vandeweerd 2016, Godden 2008, Godden et al. 2019, McGuirk und Collins 2004, Stott et al. 1979).

1.3.2. Einflussfaktoren auf die Kolostrumqualität

Es ist bekannt, dass kuhassoziierte Faktoren, wie Anzahl der Laktationen, Dauer der Trockenstehzeit, Milchlassen vor der Geburt, die Menge an Kolostrum, der metabolische Status der Kuh rund um die Kalbung oder die Eutergesundheit, die Kolostrumqualität negativ beeinflussen (Andrée O'Hara et al. 2019, Cordero-Solorzano et al. 2022, Immler et al. 2022, Puppel et al. 2020, Reschke et al. 2017, Zentrich et al. 2019). Zusätzlich muss beachtet werden, dass auch managementassoziierte Faktoren, wie die Dauer zwischen der Geburt und der ersten Melkung beziehungsweise der Fütterung des Kolostrums oder die Kolostrumaufbewahrung und die Erwärmung des Kolostrums, die Qualität stark beeinflussen können (Denholm et al. 2017, Mann et al. 2020, Morin et al. 2010, Sutter et al. 2019). Außerdem haben Umweltfaktoren, wie die Jahreszeit und der temperatur-humidity-index einen Effekt. Kuhassoziierte- und managementassoziierte Faktoren scheinen allerdings einen stärkeren Einfluss als Umweltfaktoren zu haben (Gulliksen et al. 2008, Zentrich et al. 2019).

In einer aktuellen Studie wurde das Kolostrummanagement auf österreichischen Milchviehbetrieben mittels Fragebogen erhoben. Dabei stellte sich heraus, dass der Großteil der Kälber (57,5 %) unmittelbar nach der Geburt (< 20 Minuten) von der Mutter getrennt wird und das Kolostrum in 88,1 % innerhalb sechs Stunden nach der Geburt abgemolken wird. Im Durchschnitt werden laut dieser Untersuchung innerhalb der ersten sechs Lebensstunden (91,4 %) in 70,2 % der Betriebe zwei bis vier Liter Kolostrum an die Kälber verfüttert (Hechenberger et al. 2023).

1.3.3. Möglichkeiten zur Überprüfung der Kolostrumqualität

Es gibt diverse Möglichkeiten zur Überprüfung der Kolostrumqualität. Der Goldstandard ist die Radiale Immundiffusion (RID), welche jedoch nur im Labor durchgeführt werden kann (Bielmann et al. 2010). In der Praxis sind die Brix-Refraktometrie (analog und digital), das Kolostrometer (Spindel, Hydrometer) und der ColostroCheck® Kritzinger die gängigsten Methoden. Die Brix-Refraktrometrie beruht auf Messung des Brechungsindexes des Kolostrums und zeigt laut Bielmann et al. (2010) eine starke Korrelation (r = 0,73) zur RID.

Fünfzig g IgG/l entsprechen dabei 22 % Brix (Bielmann et al. 2010, Chuck et al. 2017) bzw. 23 % Brix (Bartier et al. 2015) mit Sensitivitäten je nach Studie von 92,5 %, 90,5 % und 65,7 %, sowie Spezifitäten von 80,0 %, 85,0 % und 82,8 %. Die Temperatur spielt bei dieser Messmethode eine untergeordnete Rolle. Beim Kolostrometer hingegen wird die Dichte des Kolostrums bestimmt, die von der Temperatur beeinflusst wird. Daher ist diese Messmethode bei 20 °C validiert (Mechor et al. 1991). Das Kolostrometer teilt die Kolostrumqualität anhand verschiedener Farben ein: grün ≥ 50 g IgG/l, gelb 20-50 g IgG/l und rot ≤ g IgG/l. In einer Studie wurden ein elektronisches Refraktometer und zwei Hydrometer verglichen. Dabei lag die Sensitivität zur Auffindung schlechter Kolostrumqualität (< 50 mg/ml IgG) nach der Grenzwertoptimierung bei 75-76 %. Die Spezifität lag bei einem Hydrometer und beim Refraktometer bei 78 % und beim zweiten Hydrometer bei 66 % (Chigerwe et al. 2008).
Der ColostroCheck® Kritzinger ist ein Präzisionsdurchlauftrichter, welcher die Viskosität des Kolostrums misst und somit auch temperaturabhängig ist. Aufgrund der Zeit, die das Kolostrum für den Durchfluss braucht, können Rückschlüsse auf den Immunglobulingehalt gezogen werden. Dauert der Vorgang länger als 24 Sekunden, so kann ein Immunglobulingehalt von ≥ 50g IgG/l angenommen werden (Kritzinger 2017). In der Studie von Kritzinger (2017) wurden beim optimalen Grenzwert (23,5 sec) eine Sensitivität von 77,8 % und eine Spezifität von 73,9 % berechnet.

1.4. Immunglobulin-Gehalt im Blut des Kalbes

1.4.1. Definition „Failure of Transfer of Passive Immunity"

Wenn Kälber nicht adäquat mit qualitativ hochwertigem Kolostrum versorgt werden, kommt es zu FTPI, welcher über die Messung des IgG-Status im Blut des Kalbes bestimmt wird. Die Grenzwerte zur Definierung von FTPI variieren in der Literatur, wobei die meisten Quellen einen Grenzwert im Serum von 10 mg IgG/ml angeben (Godden 2008, Vogels et al. 2013). Andere jedoch heben den Grenzwert auf eine Serum IgG-Konzentration von 15 mg/ml an (Meganck et al. 2014).

1.4.2. Möglichkeiten zur Überprüfung des Immunglobulin-Status im Blut des Kalbes

1.4.2.1. Radiale Immundiffusion (RID)

Der Goldstandard zur Messung des IgG-Status im Serum der Kälber ist die RID. Diese Methode ist allerdings nur im Labor durchführbar und sowohl zeit- als auch kostenaufwendig, weshalb die Methode in der Praxis nur sehr wenig Anwendung findet (Godden 2008, Weaver et al. 2000). Diese Messmethode wird zur Bestimmung von Grenzwerten eingesetzt. Da eine Analyse mittels RID jedoch 18 bis 24 Stunden dauert und im Fall eines FTPI sofortiger Handlungsbedarf besteht, wird die Methode nicht oft durchgeführt. Eine Alternative sind indirekte Messmethoden, wie die Brixrefraktometrie, welche mittels Serum und Plasma der Kälber durchgeführt werden kann (Deelen et al. 2014).

1.4.2.2. Brixrefraktometrie

Die Korrelation zwischen der Brixrefraktometrie und der RID ist sehr gut (r = 0,93) (Deelen et al. 2014), r = 0,79 (Elsohaby et al. 2015), weshalb die Brixrefraktometrie als verlässliche Methode zur Überprüfung von FTPI im Feld angesehen werden kann (Elsohaby et al. 2015, Renaud et al. 2018, Zakian et al. 2018). Das Funktionsprinzip der Brixrefraktometrie beruht auf der Messung der festen Bestandteile, was der Totalproteinkonzentration entspricht.

Die Definition der Grenzwerte variiert auch hier in der Literatur sehr stark, wobei die Einteilungen basierend auf den Serum Brixwerten getroffen werden und von 7,8 % (Sutter et al. 2020, Zakian et al. 2018), 7,9 % (Gamsjäger et al. 2021), 8,4 % (Deelen et al. 2014) bis hin zu 8,7 % variieren (Elsohaby et al. 2019). Da die Grenzwerte nicht einfach zu definieren sind, werden immer öfter Kategorien zur Einteilung verwendet. Diesbezüglich hat sich jene Kategorisierung von Godden et al. (2019) durchgesetzt. Dabei werden die Serum Brixwerte in vier Kategorien unterteilt, nämlich „exzellent“ (Brixwerte ≥ 9,4 %), „gut“ (8,9 %-9,3 %), „mäßig“ (8,1 %-8,8 %) und „schlecht“ (< 8,1 %) (Godden et al. 2019).

1.4.2.3. Messung von Serum Total Proteinen

Eine weitere indirekte Messmethode stellt die Messung des Gesamtproteins im Serum (Serum Total Protein (STP)) mittels Refraktometer dar. Diese Methode zeigt eine sehr gute Korrelation mit dem Goldstandard RID von r = 0,76 (Tyler et al. 1996), r ≥ 0,88 (Wilm et al. 2018), r =

0,93 (Deelen et al. 2014) und r = 0,74 (Elsohaby et al. 2015). Die Grenzwerte bei der Messung von STP variieren von 5,2 g/dl STP (Tyler et al. 1996, Windeyer et al. 2014) bis 5,5 g/dl STP (McGuirk und Collins 2004, Tyler et al. 1996). Tyler et al. (1996) unterscheiden außerdem zwischen klinisch gesunden und klinisch kranken Kälbern. Bei gesunden Kälbern wird der Grenzwert von 5,2 g/dl STP und bei klinisch kranken Kälbern der Grenzwert von 5,5 g/dl STP empfohlen (Tyler et al. 1996). Zwischen der Brixrefraktometrie und der Messung von STP konnte eine äußerst gute Korrelation von r = 1 (Deelen et al. 2014) gezeigt werden.

1.4.2.4. Schnelltests

In Österreich ist die Blutabnahme mittels Venenpunktion den TierärztInnen vorbehalten (§4 (4) Tierärztegesetz BGBl. I. Nr. 171/2021 idgF). LandwirtInnen ist es lediglich erlaubt, die Haut mit einer Einweglanzette anzuritzen und einen Bluttropfen aus den Kapillaren zu gewinnen. Aufgrund dessen wären Schnelltests eine gute Möglichkeit, um frühzeitig Kälber mit FTPI zu identifizieren und Maßnahmen zu setzen, denn zur Durchführung eines Schnelltests würde ein Bluttropfen aus einer Kapillare reichen. Somit könnten LandwirtInnen zu jeder Zeit den Immunglobulin-Status des Kalbes prüfen. Aktuell gibt es wenig Studien, welche sich mit Schnelltests zur Identifizierung von Kälbern mit FTPI beschäftigen. Die meisten Publikationen evaluierten Kälber in einem größeren Zeitfenster, nämlich ab einem Alter von 24 Stunden bis zu einem Alter von sieben Tagen (Delhez et al. 2021), acht Tagen (Drikic et al. 2018), elf Tagen (Elsohaby und Keefe 2015) oder 15 Tagen (Stilwell und Carvalho 2011).

Im Rahmen dieser Dissertation wurde der FASTest® IgG bovine (FASTest® IgG bovine, Megacor, Österreich) evaluiert. Es handelt sich dabei um einen qualitativen lateral-flow ELISA, welcher zur Detektion von bovinen IgG im Serum, Plasma und Vollblutüberstand verwendet werden kann. Der Schnelltest hat eine Kontrolllinie und eine Testlinie, welche nur erscheint, wenn der Immunglobulingehalt weniger als 10 mg/ml beträgt. Daher wird dieser Wert als Grenzwert für FTPI in der vorliegenden Studie verwendet. Des Weiteren wurde ein Grenzwert von 8,4 % Brix im Serum angenommen, da dieser Wert einem Immunglobulingehalt von 10 mg/ml mit einer Sensitivität und Spezifität von 88,9 % entspricht (Deelen et al. 2014). Zur Durchführung des FASTest® IgG bovine wird ein Tropfen Blut (circa 20 µl Serum, Plasma oder Vollblutüberstand) in das Prüffenster des Schnelltests mit einer Einwegpipette pipettiert und drei Tropfen Pufferlösung (circa 120-150 µl) werden hinzugefügt. Nach zehn Minuten bei

20-25 °C kann das Ergebnis abgelesen werden (Megacor Diagnostics. https://www.megacor.at/useruploads/files/fastest_iggbovine_gb_web_1.pdf (Zugriff 16.11.2023)) (siehe Abb. 1).

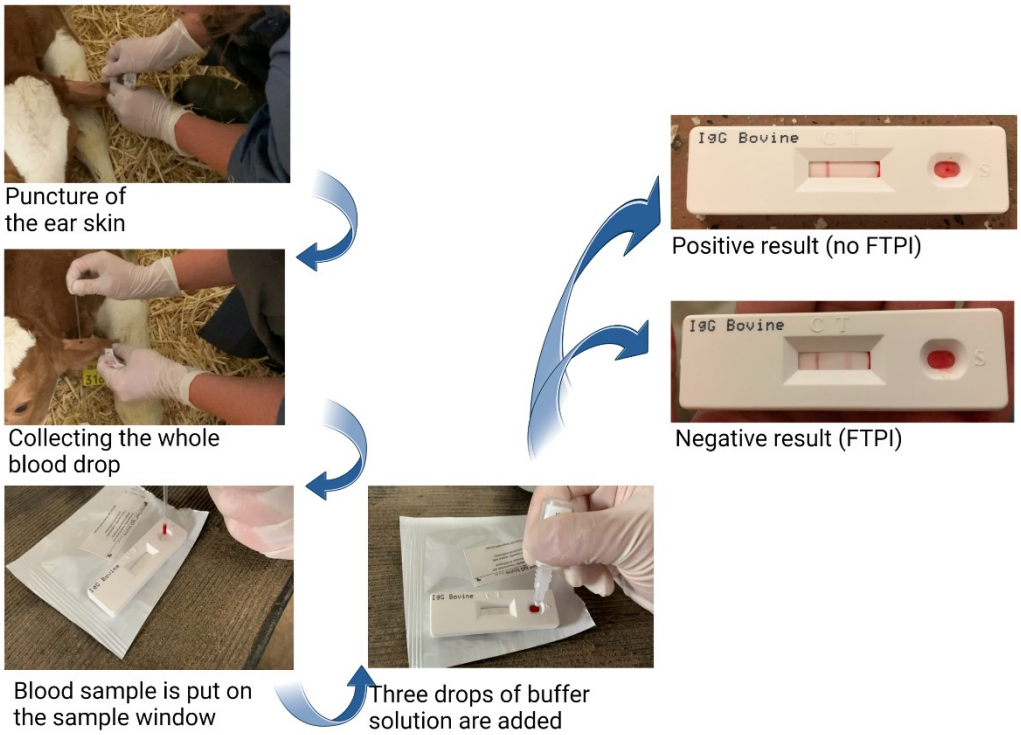

Abb. 1: Durchführung des Schnelltests zur Detektion eines Kalbes mit FTPI (FASTest® IgG bovine, Megacor, Österreich) (Hartsleben et al. 2023).

1.4.3. Einteilung auf Einzeltier- und Herdenniveau

Zur Beschreibung der Grenzwerte für FTPI auf Einzeltier- und Herdenniveaus wird die Kategorisierung nach Godden et al. (2019) verwendet. Es werden sowohl auf Einzeltier-, als auch auf Herdenniveau vier Kategorien zur Klassifizierung verwendet, welche bereits unter dem Punkt 1.4.2.2. gelistet sind und wie folgt lauten: „exzellent“, „gut“, „mäßig“ und „schlecht“ (Godden et al. 2019). Tab. 1 stellt die Grenzbereiche nach unterschiedlichen Messmethoden und die Verteilung auf Herdenniveau, beschrieben von Godden et al. (2019) dar.

Tab. 1: Unterschiedliche Grenzwerte zur Klassifizierung des Immunglobulingehalts im Serum der Kälber je nach Messmethode und erwünschte Verteilung der Kälber auf Herdenniveau (modifiziert nach Godden et al. 2019).

Kategorie	Serum IgG (g/l)	Serum Totalprotein (g/dl)	Brixwerte (%)	Erwünschte Verteilung der Kälber je Kategorie (in %)
Exzellent	≥ 25,0	≥ 6,2	≥ 9,4	> 40
Gut	18,0-24,9	5,8-6,1	8,9-9,3	≈ 30
Mäßig	10,0-17,9	5,1-5,7	8,1-8,8	≈ 20
Schlecht	< 10,0	< 5,1	< 8,1	< 10

Ziele und Hypothesen der Arbeit

Das Ziel dieser Studie war, den IgG-Gehalt im Blut der Kälber zu bestimmen, einen qualitativen lateral-flow ELISA zu validieren und den Gesundheitszustand der Kälber über die ersten 21 Lebenstage zu verfolgen.

Dabei wurden folgende Hypothesen aufgestellt:

1. Der FASTest® IgG bovine ist eine praktikable und sichere Methode bei Kälbern zwölf bis 16 Stunden nach der Geburt einen verminderten IgG-Gehalt im Vollblut festzustellen.
2. Die Anwendung des FASTest® IgG bovine aus Vollblutüberstand und Plasma zwischen dem dritten bis sechsten Lebenstag weist hoch spezifisch und sensitiv FTPI nach.
3. Im Vergleich zu Kälbern ohne FTPI weisen Kälber mit FTPI eine höhere Krankheitsanfälligkeit in den ersten 21 Lebenstagen auf.

2. Publikationen/ wissenschaftliche Beiträge als Erstautorin

Hartsleben C, Lichtmannsperger K, Tichy A, Hechenberger N, Wittek T. 2023. Evaluation of an immunochromatographic point-of-care test for the detection of failure of transfer of passive immunity in calves. Acta veterinaria Scandinavica, 65 (1): 43. DOI 10.1186/s13028-023-00707-9.

Lichtmannsperger K, Hartsleben C, Spöcker M, Hechenberger N, Tichy A, Wittek T. 2023. Factors Associated with Colostrum Quality, the Failure of Transfer of Passive Immunity, and the Impact on Calf Health in the First Three Weeks of Life. Animals : an open access journal from MDPI, 13 (11): 1740. DOI 10.3390/ani13111740.

Hartsleben C, Hechenberger N, Wanke-Jelinek PD, Wittek T, Lichtmannsperger K. 2023. Ergebnisse einer Umfrage zum Kolostrummanagement im Bundesland Salzburg [Poster]. Fortbildungsveranstaltung der ÖGT Sektion Klauentiere im Rahmen der VÖK-Jahrestagung, 23. September, 2023; Salzburg, Austria.

Hartsleben C, Lichtmannsperger K, Spöcker M, Hechenberger N, Tichy A, Wittek T. 2023. Zusammenhänge zwischen einer schlechten Immunglobulinversorgung und dem Auftreten von Kälberkrankheiten in den ersten drei Lebenswochen [Vortrag]. 17. Oberschleißheimer Wiederkäuertagung, 03. Mai – 05. Mai, 2023; Oberschleißheim, Deutschland.

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RESEARCH Open Access

Evaluation of an immunochromatographic point-of-care test for the detection of failure of transfer of passive immunity in calves

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Abstract

Background As calves are born without circulating immunoglobulin G (IgG) they depend on transfer of passive immunity via colostrum within the first hours of life. If calves are not sufficiently supplied with high qualitative colostrum they suffer from Failure of Transfer of Passive Immunity (FTPI). The objectives of this study were to evaluate a calf-side point-of-care test to detect calves with FTPI and to evaluate the cut-offs for a positive test result. Two hundred fifty calves from 11 dairy farms (born between September 2021 and September 2022) were included, whereof 23 were excluded due to incomplete data. Twelve to 16 h *post partum* the farmers carried out a point-of-care test (FASTest® IgG bovine, Megacor, Austria) using a whole blood sample. Between the 3rd and the 6th day of age, all calves were physically examined and blood samples were collected to carry out further point-of-care tests using whole blood supernatant and plasma and for measuring the Brix values in serum and plasma. Brix values in serum were used as reference for the evaluation of the point-of-care test between the 3rd and the 6th day of age, as radial immunodiffusion assays could not be conducted simultaneously.

Results Brix values were not normally distributed (median at 8.6% and 9.3% in serum and plasma). In this study, the cut-off values for the point-of-care tests using whole blood supernatant and plasma were at 8.3% Brix in serum. FASTest® IgG bovine shows high sensitivities of 90% and 84% and specificities of 70% and 72% for whole blood supernatant and plasma.

Conclusions Of the 227 investigated calves, 39.7% showed Brix values of < 8.4% (cut-off for FTPI) which indicates an urgent need to improve colostrum management. The results of the study suggest that the FASTest® IgG bovine is a suitable on-farm method to assess FTPI in whole blood supernatant and plasma of calves between the 3rd and the 6th day of age. However, the results also show that FASTest® IgG bovine is not adequate to test for FTPI using whole blood at 12 to 16 h *post partum*.

Keywords Brix, Calf-side test, Colostrum, FASTest® IgG bovine, IgG

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Background

Due to the cotyledonary synepitheliochorial structure of the bovine placenta, calves are not supplied with maternal antibodies during pregnancy and consequently they are born without circulating immunoglobulin G (IgG) [1, 2]. Therefore, calves rely on transfer of passive immunity with IgGs (~85–90% IgG1) from maternal colostrum which has to be provided during the first hours of life [1, 2]. Neonatal calves that fail to absorb sufficient colostral IgG therefore suffer from Failure of Transfer of Passive Immunity (FTPI). The threshold value for FTPI varies in literature. Many sources give a serum IgG concentration below 10 mg/mL as the cut-off value [3, 4]. Others, however, raise the cut-off value up to a serum IgG concentration of 15 mg/mL [5]. FTPI is known as a significant problem that can lead to early calf losses due to gastroenteritis, pneumonia or septicaemia [3, 6, 7]. Besides high economic losses and reduced profitability, the increased morbidity and mortality rates pose a major animal welfare issue [8–12]. A study from Switzerland investigated 373 dam-calf pairs, whereof 162 (43.5%) of the calves showed FTPI [13]. It has been reported that the probability of a low serum immunoglobulin concentration in calves increases significantly (odds ratio = 10.7), if the colostrum contains less than 50 g/L IgG [13]. Preliminary results from a project by the Austrian Animal Health Service on the evaluation of colostrum management on dairy farms show that 49.8% of the investigated colostrum samples showed Brix values of less than 22% [14]. Besides high colostrum quality the time between parturition and colostrum delivery to the neonatal calf plays an essential role, since the absorption of immunoglobulins in the intestine decreases within the first 24 h of life and completely ceases at 24 to 36 h. In summary, timing of colostrum feeding after parturition (within 2 h), colostrum quality (≥50 g/L IgG) and the amount of colostrum fed to the calf (>4 L) within the first hours of life are the most important factors to prevent FTPI [1, 3, 15–17]. Furthermore, the efficiency of immunoglobulin absorption tends to be higher in colostrum with low bacterial contamination [18]. In an Austrian online questionnaire on calf management, only 20.8% of the farmers had a colostrum testing protocol, and of these, 86.1% based the protocol on visual inspection [19]. It is essential for farmers to be able to evaluate the success of passive immunity transfer at the herd-level to improve their colostrum management [20, 21]. Although there are various direct methods to assess the immunoglobulin concentration in calves they are rarely used in practice since they are typically time consuming and expensive. The radial immunodiffusion (RID) assay is the gold standard method to measure the quantity of IgG in calf serum [3, 22]. This method has to be performed by laboratory technicians and takes 18 to 24 h. A common alternative is to use indirect methods such as the Brix refractometer using calf plasma or serum [23]. Since the correlation between the measurement by Brix refractometry and the RID is good (r = 0.93) [23], Brix refractometry can be considered a reliable method to directly identify FTPI under field conditions [24–26] by measuring the total solids which approximates to the total protein concentration. The serum Brix measurements of the calves can be categorized using the thresholds described elsewhere [1, 23, 26–29]. Cut-off values to detect FTPI by measuring Brix percentage in serum vary from 7.8% [26, 28], 7.9% [29], 8.4% [23] to 8.7% [27]. Because of these variations, the Brix measurements can be divided into the four categories "excellent" (Brix level ≥ 9.4%), "good" (8.9–9.3%), "fair" (8.1–8.8%) and "poor" (< 8.1%) [1], which can also be used on a herd-level. Blood samples are obviously required to assess the immunoglobulin concentration in serum or plasma. In Austria taking blood samples puncturing a vein is restricted to veterinarians but farmers are allowed to scarify the skin producing a blood drop from capillaries. Therefore, indirect methods such as the Brix refractometer are rarely used in practice. It is frequently not practical to take a venous blood sample in the first hours of the calf's life. However, a commercially available point-of-care test could be used to assess immunoglobulin concentrations by farmers since only a few microliters of blood are required for the procedure. To the best of our knowledge, there has been limited research been done on testing the transfer of passive immunity of neonatal calves 12 to 16 h after birth [30]. In the majority of studies, calves from 24 h of age until 7 [31], 8 [32], 11 [33] or 15 days of age [34] were studied. To implement a reliable tool to assess the IgG status of the neonatal calf, an early, inexpensive and practical tool such as the FASTest® IgG bovine (FASTest® IgG bovine, Megacor, Austria) might be used. The point-of-care test is a qualitative test for the detection of bovine IgG in serum, plasma or whole blood supernatant. According to the manufacturer's specifications, the point-of-care test shows a negative result if the immunoglobulin concentration is less than 10 mg/mL and the FASTest® IgG bovine is licensed to be used in calves from 24 h up to 7 days of life.

The objectives of this study were to evaluate the feasibility of a calf-side point-of-care test carried out at different times *post partum* (*pp*) to detect calves with FTPI and to evaluate the cut-offs for a positive test result. Since the point-of-care test is not approved for whole blood, the results with this medium were assessed by comparing with the results of point-of-care tests carried out with whole blood supernatant and plasma.

We hypothesized (1) That the threshold of the point-of-care test to indicate FTPI is 8.4% Brix in serum (2) That the results of the point-of-care tests carried out 12 to 16 h *pp* and the point-of-care test carried out 3 to 6 days

pp are strongly associated indicating that the early measurement has a sufficient diagnostic value. Therefore, the results of the point-of-care tests with whole blood, whole blood supernatant and plasma were compared.

Methods

Ethical consideration

This study was approved by the Ethics and Animal Welfare Committee (ETK) of the University of Veterinary Medicine, Vienna and the Austrian national authorities, according to § 26 of the Tierversuchsgesetz 2012 – TVG 2012 (GZ.: 2021–0.644.875).

Study farms and animals

Two hundred and fifty calves from 11 dairy farms in the region of Enns-Pongau and Lungau (federal state of Salzburg, Austria) born between September 2021 and September 2022 were included (see Fig. 1). The calves were included in the study on the sequence of their birth and excluded only, if they were extremely stressed, uncooperative or died within the first 6 days of life (n=0). Seven farms already participated actively in a previous project by the Austrian Animal Health Service on the evaluation of colostrum management in dairy farms. A part of this larger investigation has been published previously [14]. The remaining 4 farms joined the study on their own intention as they could provide additional samples conveniently. In total, 227 female and male calves aged up to 6 days of age were finally involved after removing the data of 23 calves due to missing information. The breed was primarily Fleckvieh (Simmental) (n=118 with 59.3% female and 40.7% male calves), Pinzgauer (n=65 with 33.8% female and 66.2% male calves) and cross-breeds (Belgian Blue) (n=44 with 45.5% female and 54.5% male calves). All calves were separately from their dams within one hour and the farmers took care that suckling was not possible. Of the 227 calves included, 217 calves received colostrum from their own dam and 10 calves received non-maternal colostrum (pooled frozen colostrum, fresh colostrum from another cow than mother). Two hundred and eight calves received their first meal within 4 h *pp*. Nineteen calves received the colostrum after ≥4 h. Of these, 17 calves initially showed no suckling reflex and 2 calves were fed later since the farmer did not manage to deliver the colostrum within 4 h. One hundred and eighty-seven calves had a colostrum intake of ≥2 L whereas 40 calves had a colostrum intake of less than two liters. All calves were in barns and they only received colostrum and transition milk within the first 6 days *pp*. The calves were fed twice daily under supervision of the farmers. Twelve calves were fed several times a day because their general condition and sucking reflex were poor. Factors that were associated with colostrum quality, FTPI and their impact on health events in the first three

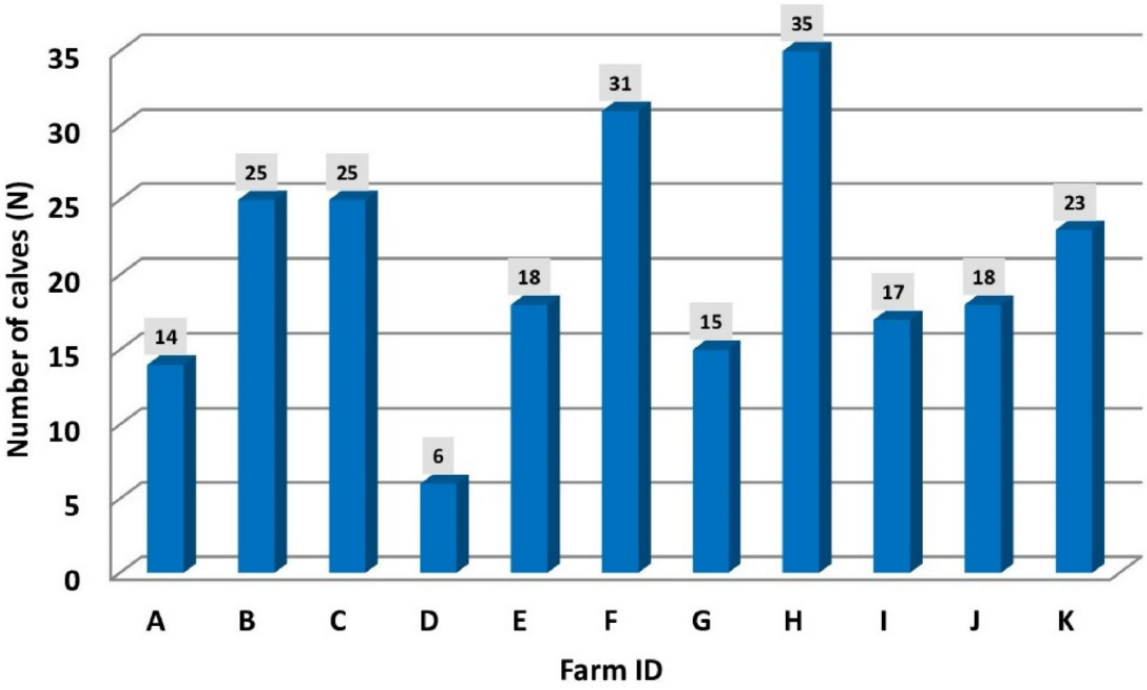

Fig. 1 Total number of included male and female calves per farm. In total, 227 calves originating from 11 dairy farms participated in the study

weeks of life was also evaluated and already published [35].

Point-of-care test implementation at 12 to 16 h post partum by farmers

Prior to the study, all 11 dairy farms were visited by one author (CH) and the farmers were trained using the point-of-care test system (FASTest® IgG bovine, Megacor, Austria) (Fig. 2). The FASTest® IgG bovine is a qualitative test for the detection of bovine IgG in serum, plasma or whole blood supernatant. The point-of-care test includes a control line and a test line. According to the manufacturer's specifications, the test line solely appears, if the immunoglobulin content is less than 10 mg/mL (FTPI). Twelve to 16 h *pp*, the point-of-care test was carried out by the farmers as described (Fig. 2). The skin of right or left ear edge was scarified using a hypodermic needle. Subsequently, the whole blood drop (approximately 20 µL) was collected using a plastic pipette and the test was carried out according to the manufacturers specifications. Briefly, the blood sample was put on the sample window of the test cassette and three drops of buffer solution (approx. 120 to 150 µL) were added to the sample window. After 10 min at 20 to 25 °C the test kit was read by the farmers. The test results were recorded on paper and a digital photograph was taken of each test result. Subsequently, the photograph was sent to the principal author (CH) for further review of the test result. It is common to feed transition milk of the mother to the calves for 3 days. Farmers were instructed to carry on with their herd-specific management regardless of the point-of-care test result.

Point-of-care test implementation at 3 to 6 days of age by the principal author (CH)

EDTA and serum samples were collected by the principal author (CH) from calves between 3rd and 6th day of age by jugular venipuncture using an 18-gauge needle and vacutainer tubes (Vacuette®, Greiner Bio-One GmbH, Austria). The point-of-care tests were performed using two different samples: whole blood supernatant and plasma. To receive whole blood supernatant, EDTA blood samples were left untouched in an upright position for 5 min at 20 to 25 °C. Plasma and serum samples were

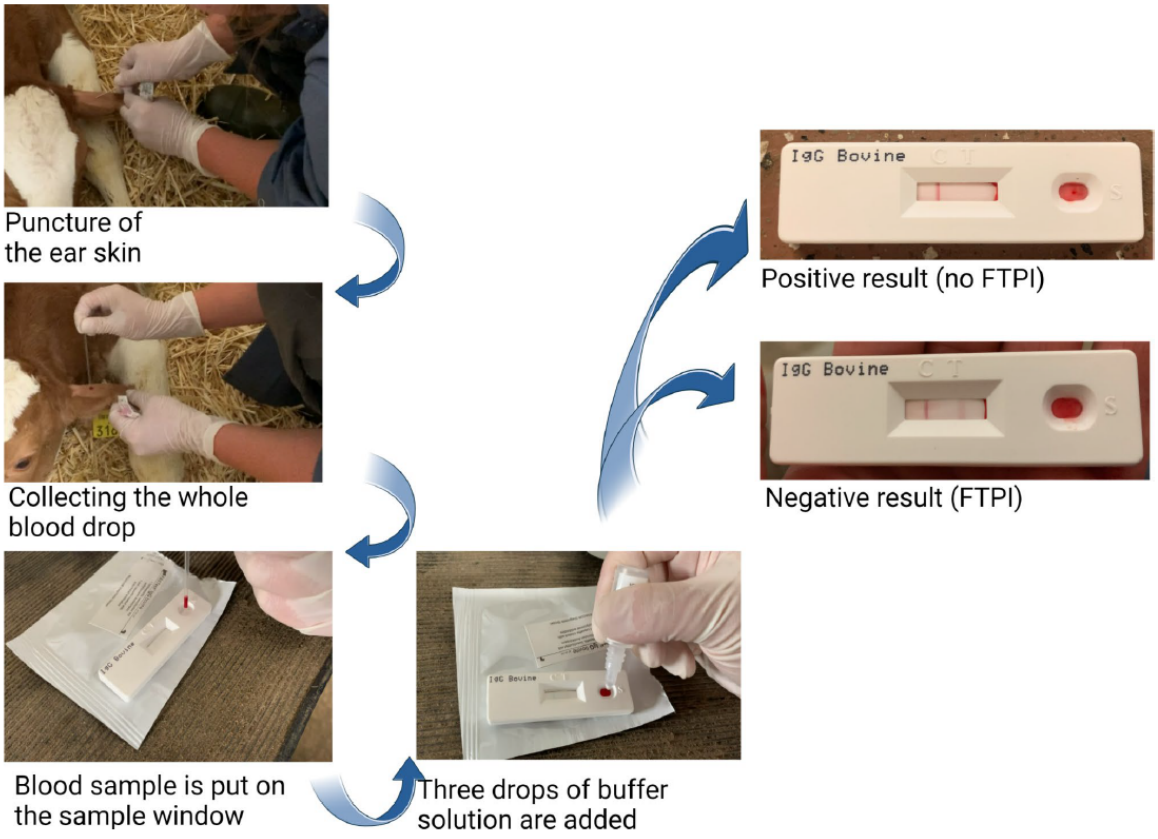

Fig. 2 All 11 farms were visited by the principal author and the farmers received a training on the usage of the point-of-care test (FASTest® IgG bovine, Megacor, Austria) for the detection of Failure of Transfer of Passive Immunity (FTPI). The farmers draw the samples 12 to 16 h *post partum*

produced by centrifugation at 1,500 g for 10 min at 20 to 25 °C on the farms (CGOLDENWALL 800D Electric Centrifuge Medical Lab Centrifuge 4,000 rpm with CE 6×20 mL, Zhengzhou Jin Chen Electronic Technology Co. Ltd., China). All tests were carried out according to the manufacturer's specifications under field conditions (Fig. 3).

Reference method: brix refractometry

Serum and plasma Brix values were determined immediately after sample collection under field conditions (approx. 20 to 25 °C) on each farm using a digital Brix refractometer (MA871 Refractometer, Hebesberger, Austria) (Fig. 3). The Brix refractometer (0 to 85% Brix) was calibrated using deionized water. Calibration was carried out routinely at the beginning of the analysis on each farm and following the measurement of 10 serum or plasma samples. After calibration, serum or plasma was pipetted onto the prism using a one-way 2 ml plastic pipette. The Brix percentage was recorded twice and the mean value was used for statistical analysis. Aliquots were made in 1.5 mL Eppendorf tubes® (Eppendorf®, Eppendorf Austria GmbH, Austria) and transported on ice in a polystyrene box. All samples were frozen within two hours at minus 18 °C.

Statistical analysis

Descriptive and explorative statistical analysis was performed using Microsoft Excel 2010 (Microsoft®, Washington, USA) and IMB® SPSS® Statistics Version 28 (IBM®, New York, USA). The serum and plasma Brix percentages of the calves were categorized using the threshold described elsewhere (excellent=≥9.4%, good=8.9–9.3%, fair=8.1–8.8%, poor=<8.1%) [1]. For each calf three point-of-care tests were performed: One by the farmer (whole blood) and two by the principal author (whole blood supernatant and plasma) (see Fig. 3). Statistical analysis was performed in two stages: Primary, test validity of point-of-care tests with whole blood supernatant and plasma using the BRIX values in serum as reference was assessed using the Youden-Index and ROC-analysis. The Youden index, which is calculated from the sum of sensitivity and specificity minus 1, to calculate the optimum limit value. The area under the receiver operating

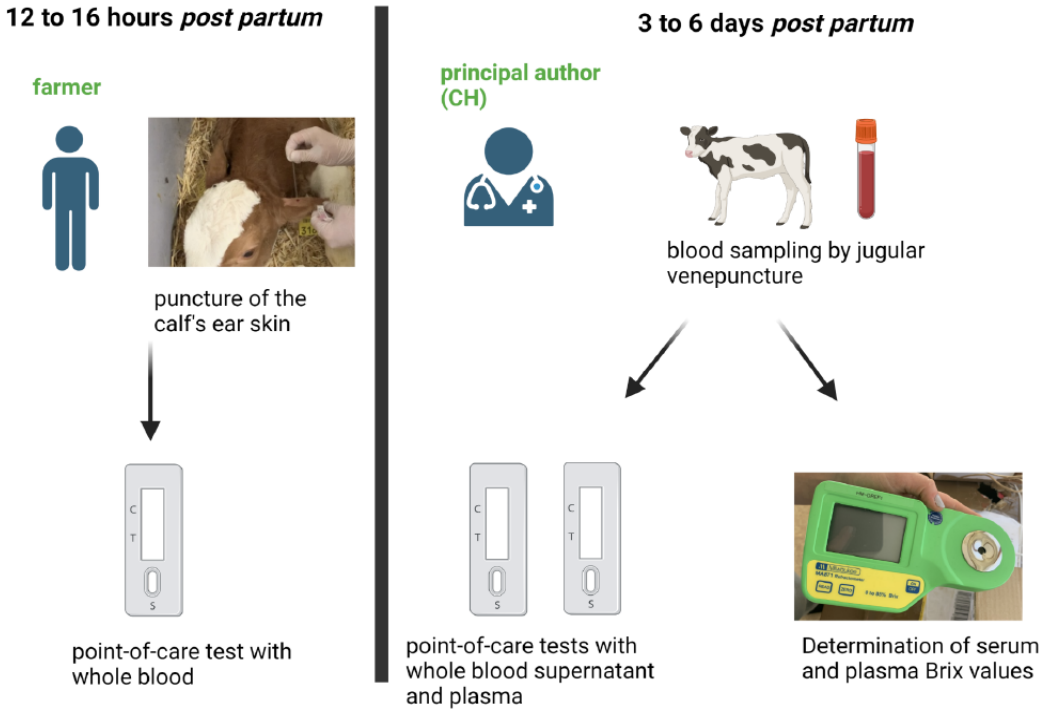

Fig. 3 The samples were collected 12 to 16 h *post partum* (whole blood) by the farmers and 3 to 6 days *pp* (EDTA and serum) by the principal author (CH). All calves were clinically examined at the time of sampling. The figure illustrates the steps from sample collection to sample analysis

characteristic (ROC) curve (AUC) was included as a measure of the quality of the test. The AUC values range between 0.5 and 1.0, where the higher values indicate better quality. Second, the results of the three different point-of-care tests for each individual calf were compared to each other. There were three comparisons: Firstly, the comparison of the results of the farmer's point-of-care tests using whole blood with the results of the point-of-care tests with whole blood supernatant, carried out by the principal author. Secondly, the results of the farmer's point-of-care tests using whole blood were compared to the results of the point-of-care tests with plasma. Thirdly, the results of the point-of-care tests with whole blood supernatant were compared to those with plasma (both carried out by the principal author). Definition of a negative point-of-care test result was if the calf showed FTPI (IgG less than 10 mg/mL according to the manufacturer's specifications which equals 8.4% Brix in serum according to previous investigations [23]). For this purpose, cross tables were created and the Cohen's Kappa (κ) was used. Cohen's Kappa was calculated based on the comparison of point-of-care tests and describes the agreement between them. Kappa values can range from 0 to 1 and they are interpreted as follows: ≥0.81 very good agreement; 0.61 to 0.8 good agreement; 0.41 to 0.6 moderate agreement; 0.21 to 0.4 fair agreement; and ≤0.2 poor agreement [36]. Using cross tables, sensitivity and specificity of the point-of-care test comparisons were calculated. All tests were calculated with a significance level of $P<0.05$. Tests of normality were carried out using Kolmogorov-Smirnov test.

Results

Farms and calves

In total 227 calves originating from 11 farms were included. IgG levels based on the measurements of the calves' serum were categorized as poor, fair, good and excellent [23] (Fig. 4). The proportion of calves that had low levels of IgG varied greatly across the farms.

Brix values

The intra- and inter-reliabitity for the digital Brix refractometry was not calculated in the present study as the accurate assessment [37]. According to the Kolmogorov-Smirnov-test, P-value for Brix values in serum was <0.001 and for Brix values in plasma 0.002, which means that data were not normally distributed. Regarding the Brix values in serum, there was a minimum of 6.3% and a maximum of 11.8%. Percentiles (10th, 25th, 75th, 90th) were at 7.6%, 8.0%, 9.3% and 9.9% respectively. The median was at 8.6% with a variance of 0.9%. For Brix values in plasma the minimum was at 7.0% and the maximum at 12.4%. The percentiles in this case were at 8.4%, 8.7%, 9.3%, 9.9% and 10.5%. A median of 9.3% with a variance of 0.8% was calculated.

In total, 137 of the calves (60.3%) and 90 of the calves (39.7%) showed serum Brix values of ≥8.4% and serum Brix values of <8.4%, respectively (Fig. 5). Consequently, 39.7% of the calves were classified as suffering from FTPI. The statistical measures varied between the individual farms. Further details are published elsewhere [35].

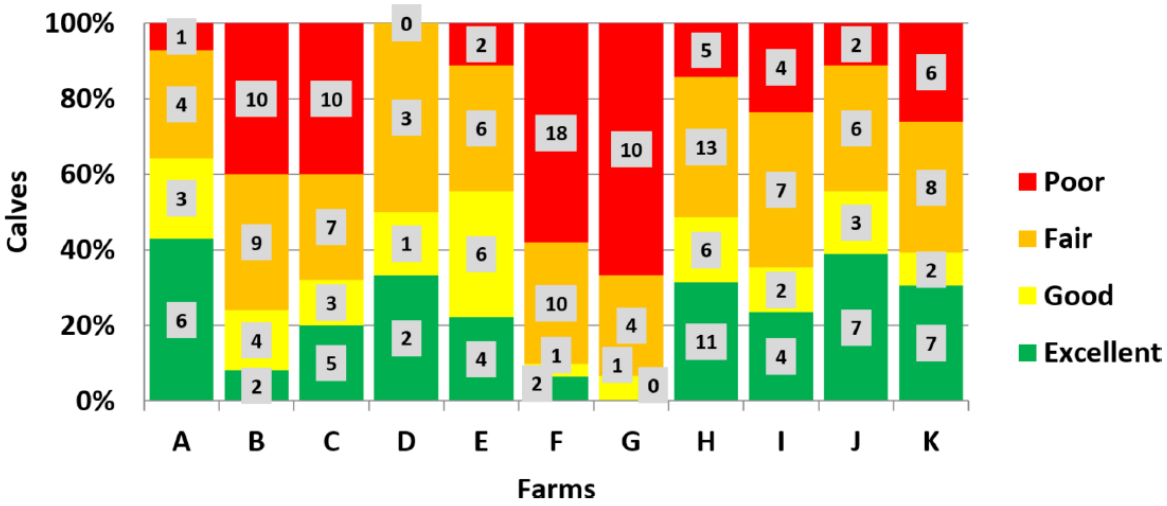

Fig. 4 Summary of the four categories for immunoglobulin G levels based on the BRIX measurements in serum on the 11 included farms according to Godden and colleagues (2019)

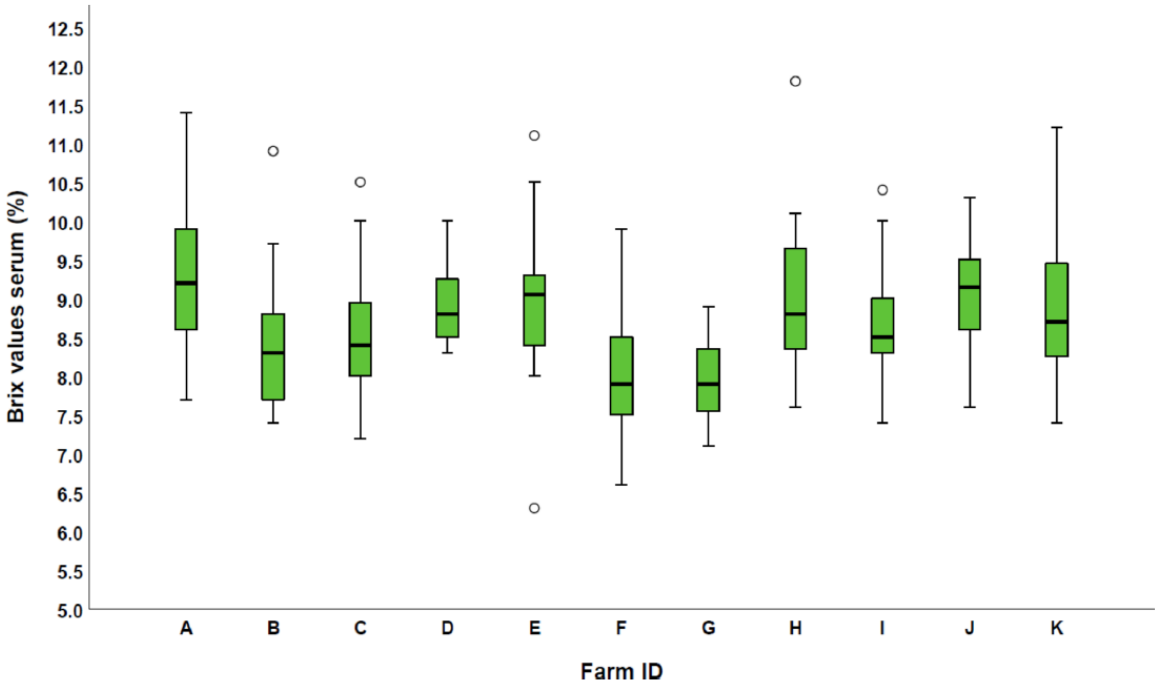

Fig. 5 Boxplots of the Brix values in serum for each individual farm (n = 11) and 227 investigated calves

Receiver operating curves

Evaluating cut-offs for the point-of-care test carried out by the principal author using whole blood supernatant

The point-of-care test using whole blood supernatant carried out by the principal author between 3rd and 6th day *pp* was carried out using 137 'no FTPI' and 90 FTPI samples. In this case, the AUC was 0.84 and 0.88 when using the Brix values from serum and the Brix values from plasma (collected between the 3rd and the 6th day of age). The optimal cut-off was set at 8.3% Brix for serum (SE = 0.90; SP = 0.70) and 9.2% Brix for plasma (SE = 0.79; SP = 0.80). The Youden Index was 0.60 and 0.59 for serum and plasma, respectively (Fig. 6).

Evaluating cut-offs for the point-of-care test carried out by the principal author using plasma

The point-of-care test using plasma carried out by principal author between 3rd and 6th day *pp* was carried out on 153 'no FTPI' and 74 FTPI test results. The AUC was 0.81 and 0.81 when using the Brix values from serum and the Brix values from plasma (collected between the 3rd and the 6th day of age), respectively. The optimal cut-off was set at 8.3% Brix for serum (SE = 0.84; SP = 0.72) and 8.9% Brix for plasma (SE = 0.87; SP = 0.68). The Youden Index was 0.56 and 0.55 for serum and plasma, respectively (Fig. 6).

Point-of-care test comparison

In order to know if the point-of-care test 12 to 16 h *pp* and the point-of-care tests between 3rd and 6th day *pp* are strongly associated the results of all 3 point-of-care tests were compared to each other (Table 1).

Comparison of point-of-care test results from whole blood (12 to 16 h post partum) and whole blood supernatant (3 to 6 days post partum)

Of the 227 included calves 132 calves (60.4%) had a sufficient immunoglobulin concentration and 90 calves (39.6%) had an insufficient immunoglobulin concentration according to the test result after 3 to 6 days using the whole blood supernatant samples. Of the 185 (100%) point-of-care test results showing 'no FTPI' gained after 12 to 16 h by the farmer, 132 (71.4%) were correctly identified as 'no FTPI' and 53 (28.6%) were incorrectly identified as 'no FTPI' (Table 2). The sensitivity and specificity in this comparison were 96.4% and 41.1%, respectively. The kappa coefficient was 0.412 (0.300; 0.525), indicating a moderate agreement (see Table 1).

Comparison of point-of-care test results from whole blood (12 to 16 h post partum) and plasma (3 to 6 days post partum)

Of the 227 included calves, 153 calves (67.4%) showed 'no FTPI' and 74 calves (32.6%) had an insufficient immunoglobulin concentration according to the test result after

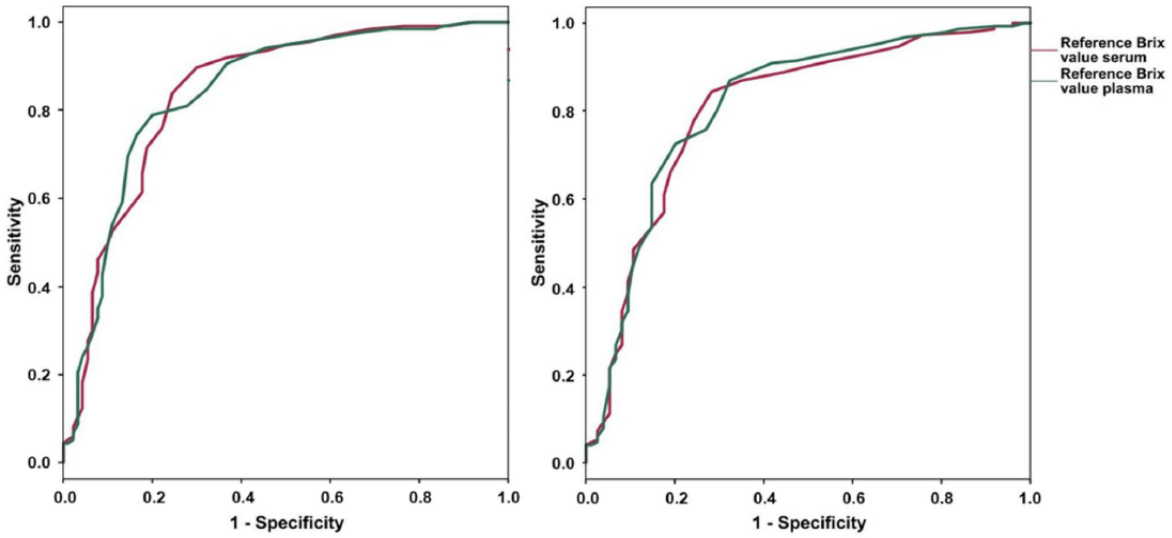

Fig. 6 Receiver Operating Curve for the point-of-care test (FASTest® IgG bovine, Megacor) using whole blood supernatant (left) and plasma (right) carried out 3 to 6 days *post partum*. The Brix values from serum and plasma were used as reference to calculate the optimal threshold

Table 1 Comparison of the three point-of-care tests carried out for each individual calf

Point-of-care test	Comparison test	PPV (95% CI)	NPV (95% CI)	SE (95% CI)	SP (95% CI)	K (95% CI)
Whole blood*	whole blood supernatant#	71.4% (64.6%; 77.5%)	88.1% (76.1%; 95.6%)	96.4% (92.3%; 98.7%)	41.1% (31.3%; 51.4%)	0.412 (0.300; 0.525)
Whole blood*	plasma#	76.2% (69.7%; 82.0%)	71.4% (56.8%; 83.5%)	92.2% (87.2%; 95.7%)	40.5% (29.8%; 51.9%)	0.368 (0.240; 0.496)
Whole blood supernatant#	plasma#	98.5% (95.6%; 99.8%)	80.0% (71.0%; 87.4%)	88.2% (82.5%; 92.7%)	97.3% (91.9%; 99.5%)	0.81 (0.732; 0.889)

This table gives an overview of the comparison of the three point-of-care tests carried out for each individual calf. As there was no RID implemented, the three point-of-care tests were compared to each other in order to calculate these values. Positive predictive value (PPV), negative predictive value (NPV), sensitivity (SE), specificity (SP) and Cohen's Kappa (K) of one point-of-care test (FASTest® IgG bovine, Megacor) using different blood collecting time points *pp* (*12 to 16 h *pp* by the farmers; #3 to 6 days *pp* by the principal author) and different media. The lower and upper 95% confidence intervals are given in parentheses (95%CI).

3 to 6 days using the plasma samples. Of the 185 (100%) point-of-care test results gained after 12 to 16 h by the farmer, 141 (76.2%) were correctly identified 'no FTPI' and 44 (23.8%) were incorrectly identified as 'no FTPI' (Table 2). The sensitivity and specificity in this comparison were 92.2% and 40.5%, respectively. The kappa coefficient was 0.368 (0.240; 0.496), indicating a fair agreement (see Table 1).

Comparison of point-of-care test results from whole blood supernatant and plasma (3 to 6 days post partum)

As described in the manufacturer's specifications, the point-of-care test using plasma after 3 to 6 days *pp* was implemented as the reference test for comparison. Two hundred and twenty seven calves were included. Of these calves, 137 calves showed 'no FTPI' and 90 calves were categorized as having FTPI, according to the point-of-care test with whole blood supernatant. Of the 137 calves that showed "no FTPI", 135 were correctly and 2 were incorrectly identified as having "no FTPI" in comparison to the point-of-care test with plasma (Table 2). The sensitivity and specificity in this comparison were 88.2% and 97.3%. The kappa coefficient was 0.81 (0.732; 0.889), indicating a good agreement (Table 1).

Discussion

Occurrence of FTPI

In order to protect calves against FTPI, adequate supply of high qualitative colostrum is necessary. Calf-side point-of-care tests would be a suitable on-farm method to detect calves with FTPI with some limitations. According to the manufacturer's specifications of FASTest® IgG

Table 2 Cross tabulation on the point-of-care test results

		FASTest® IgG bovine with whole blood supernatant (3 to 6 days *p.p.*)		
		no FTPI	**FTPI**	**total**
FASTest®IgG bovine with whole blood (12 to 16 h*p.p.*)	no FTPI	132	53	185
	FTPI	5	37	42
	total	137	90	227
		FASTest®IgG bovine with plasma (3 to 6 days*p.p.*)		
		no FTPI	FTPI	total
FASTest®IgG bovine with whole blood (12–16 h*p.p.*)	no FTPI	141	44	185
	FTPI	12	30	42
	total	153	74	227
		FASTest®IgG bovine with plasma (3–6 days*p.p.*)		
		no FTPI	FTPI	total
FASTest®IgG bovine with whole blood supernatant (3–6 days*p.p.*)	no FTPI	135	2	137
	FTPI	18	72	90
	total	153	74	227

This table shows a cross tabulation on the point-of-care test results carried out by the farmers (12 to 16 h *post partum (pp)*) and by the principal author (3 to 6 days *pp*) using different media for the test

bovine, the cut-off value is set at 10 mg/mL which has been described to equal 8.4% Brix in serum with a sensitivity and specificity of 88.9% [23]. Different Brix values have been estimated to correspond to a serum IgG concentration of 10 mg/mL [1, 23, 26–29]. It has to be stressed that studies have used different reference tests to determine an optimal cut-off. For example, serum total protein (STP) or enzyme-linked immunosorbent assays are used as comparison methods to implement a value corresponding a serum IgG concentration of 10 mg/mL [26]. RID as gold standard would be optimal as a reference comparison [23]. Therefore, we hypothesized that the threshold of the point-of-care test indicating FTPI is 8.4% in serum [23], as it has been described that a serum IgG concentration of 10 mg/mL equals 8.4% Brix in serum with a high sensitivity and specificity [23]. Furthermore, there was a high correlation of Brix percentage and IgG (analyzed by RID) of r = 0.93 [23]. In general, it has to be emphasized, that one single cut-off value is not adequate to categorize calves with FTPI on a herd-level. It would be beneficial to divide the serum brix values of the calves into categories, for instance as described by Godden and coworkers [1]. A dichotomous approach (FTPI yes or no) is possible for instance by using such a point-of-care test. However, on a herd-level the categorization would be beneficial since it should be emphasized that calves do not just have an IgG level of > 10 mg/mL, they should have an excellent colostrum supply with > 25 mg/mL. In the present study, however, there had to be a limit value based on the validity of the point-of-care test (yes/no).

This applies not only to the cut-off value determination of Brix percentage, but also to the cut-off value determination of other units, such as serum IgG concentration. Regarding this, cut-off values range from 10 mg/mL [3, 4] to 15 mg/mL [5]. Further studies are needed to clarify this issue.

Brix percentages and their cut-off values

In total, 90 calves (39.7%) showed serum Brix percentages of < 8.4%, respectively. In other studies, the number of calves with FTPI was at 27% [28], 13% [26], 8.3% [29], 4.75% [23] and 43.3% [27]. These great differences are due on the one hand to the different cut-offs, and on the other hand, there are both, geographical- and management-related differences. Furthermore, parameters such as season, temperature, time of sampling and cattle breed can have an effect on the results. In the present study, there are differences between the individual farms. Further studies and a greater study population are necessary to show the impact of these parameters on Brix values.

Brix cut-off values in serum were at 8.3% for point-of-care tests using plasma and whole blood supernatant. According to the ROC analysis, sensitivity and specificity were at 84.3% and 71.6% for point-of-care tests with plasma and at 89.8% and 70.0% for point-of-care tests with whole blood supernatant, respectively. Compared to an investigation carried out on dairy calves, the calculated cut-off values of the present study were lower, possibly explaining the high number of false results ('no FTPI') [23]. Assuming the calculated cut-off values in the point-of-care test were higher, more calves would show FTPI, as discussed elsewhere [5].

Brix cut-off values in plasma were also investigated. The cut-offs in this respect were at 8.9% for point-of-care tests with plasma and 9.2% for point-of-care tests with whole blood supernatant, respectively. There are multiple studies showing a substantial difference between serum and plasma Brix levels of 8.7% Brix in serum and 9.4% Brix in plasma and 7.8% Brix in serum and 8.6% Brix in plasma [27, 28]. The cut-off results of the present investigation were within the range of the aforementioned studies. One of the potential explanations for cut-offs in plasma being higher than in serum might be because plasma contains coagulation proteins such as fibrinogen, which is soluble and clots during serum processing [38]. Furthermore, EDTA (used in this study) as well as other anticoagulants (lithium-heparin, citrate or heparin) can incorrectly lead to an increase of plasma total protein concentrations due to an incorrect ratio between blood and the anticoagulant [39].

It has been proven elsewhere that Brix values of serum samples show higher agreement with RID than

those from plasma with an accuracy of 79.7% in serum and 74.7% in plasma, respectively [27]. A digital Brix refractometer has been implemented as reference in the present study. Currently, the RID is recognized as gold standard in detecting immunoglobulins in bovine serum samples. Multiple investigations showed that the RID results and the Brix results show a good accuracy. Therefore, it has to be stressed that using the Brix values as reference was suboptimal. With regard to the Brix values in serum on days 3 to 6 of life, 90 calves (39.7%) had FTPI, if a threshold of 8.4% was used [23].

Point-of-care test comparison

The second hypothesis was that the results of the point-of-care tests carried out 12 to 16 h *pp* and the point-of-care tests carried out 3 to 6 days *pp* give the same test result (FTPI yes or no) indicating that the early measurement has sufficient diagnostic value. Since the point-of-care test is not approved for whole blood, the results with this medium were checked by comparing with the results of point-of-care tests carried out with whole blood supernatant and plasma. It was feasible to carry out the point-of-care test using whole blood from the calf's ear. Nevertheless, the point-of-care test showed a poor performance in terms of false positive and false negative rates in comparison to the tests carried out after 3 to 6 days of age. It needs to be stressed that no reference test (Brix value) was available for this time point. In future investigations, the gold standard also needs to be carried out in parallel.

In total, 185 calves were identified as having 'no FTPI' by the point-of-care test using whole blood after 12 to 16 hours *pp* (by the farmers). Of these 185 calves, 28.7% (53) and 23.8% (44) of the calves had been identified incorrectly using whole blood supernatant and plasma as the reference test for comparison at 3 to 6 days of age. The high number of false "no FTPI' results make the farmers believe that the new-born calves are supplied sufficiently with IgG. The FASTest® IgG bovine has a low specificity 12 to 16 h *pp*, which might be due to any kind of cross reaction between the whole blood cells and the antibodies of the lateral flow ELISA. The exact causes seem to be unknown.

In total, 42 calves have been identified as having FTPI by the farmers' point-of-care test with whole blood. Of these 42 calves, (11.9%) 5 (comparison test with whole blood supernatant) and 28.6% (12) (comparison test with plasma) had been identified incorrectly. One of the major limitations of the study was, that there was no reference test (digital Brix refractometry) carried out 12 to 16 h after birth. In brief, it can be summarized that the collection of whole blood from the calf's ear using a capillary was feasible but the timing and the sample type do not seem to be suitable for FASTest® IgG bovine.

Additionally, the qualitative point-of-care test solely divides the calves into the ones having FTPI and the ones not having FTPI. It is well known that the IgG status of the calves should not just be divided dichotomously since there is a difference in morbidity and mortality rates between calves having poor (<8.1% Brix in serum), fair (8.1–8.8% Brix in serum), good (8.9–9.3% Brix in serum) or excellent (≥9.4% Brix in serum) TPI [1]. Therefore, it needs to be further investigated if the point-of-care test is an economically useful investment taking into account the information you receive and the conclusions you can draw from the results.

Point-of-care tests with whole blood supernatant and plasma compared to each other show a very good agreement with a kappa value of 0.81 (ranging from 0.73 to 0.89). The ZAPvet Bovine IgG (ZAPvet Bovine IgG test, ZBx Corp., Toronto, ON, Canada), which is a different calf-side point-of-care test, shows a sensitivity of 82.0% and a specificity of 65.0% [33]. Sensitivity as well as specificity are significantly lower for ZAPvet Bovine IgG than for FASTest® IgG bovine regardless the sample used. FASTest® IgG bovine has a sensitivity of 90% and 84% and a specificity of 70% and 72% for whole blood supernatant and plasma, respectively.

General limitations

There were some major limitations in the present study. The used reference method was not the gold standard (RID). There is good evidence showing that there is a high correlation between the RID and Brix refractometry [23, 27–29]. Since there is an accuracy of almost 80% between Brix values in serum and RID, this value was used as the reference method [27]. The digital Brix refractometer was used as a fast and cost effective reference method, which can be carried out in the local veterinary practice without any special skills or equipment. Nevertheless, it has to be mentioned that even this method is not cheap. Initial cost may range from $US 200–400 and an annual calibration has to be done. With regard to the calculation of sensitivity and specificity, it has to be emphasized at this point that their calculation would have been preferable using the gold standard method (RID).

As it was not feasible for the principal author (CH) to visit each calf and take a blood sample 12 to 16 h *pp*, due to night parturition for example, farmers were also involved in sample collection. Since the national law allows farmers only to draw capillary blood, whole blood was used even though FASTest® IgG bovine is approved for whole blood supernatant, plasma and serum. According to the manufacture's specifications, the test time slot for FASTest® IgG bovine is from 24 h up to 7 days *pp*. In future investigations, additional serum and plasma samples have to be drawn from the calves 12–16 h *pp* to describe whether the cross-reactivity of the blood

constituents with the antibodies or other factors influence the test results. Another aspect is that the value of IgG may still be increasing at this time point. For example, if a calf did not drink colostrum until 6 h *pp*, there is a high probability of having a test result with FTPI 12 h *pp*. However, since TPI is not yet complete at this time, the same calf might not have FTPI at a later time point of measurement. Another aspect is that providing additional colostrum even 24 h *pp* is beneficial and might increase IgG levels [30, 40]. So, if the point-of-care test had been done at a later time point (for example 18–24 h *post par,tum*), better matches to the results of the tests performed between 3rd and 6th day might have been achieved. Furthermore, Brix refractometry or even better RID should be done at 12 to 16 h *pp* in order to check the presented values for FASTest® IgG bovine at this timeslot.

The 11 farms are a good representation of the region of Enns-Pongau and Lungau (federal state of Salzburg, Austria). In order to get a reliable overview on the actual colostrum supply of calves in the province of Salzburg, further studies are necessary. To the best of our knowledge, this was the first attempt to focus on the feasibility of a calf-side point-of-care test in Austria. Further studies are needed to investigate if capillary whole blood is a reliable medium to test for FTPI in calves at different time points with calf-side point-of-care tests. This study was solely a small scale investigation in a defined region in Austria. To receive data on the true prevalence of FTPI in Austria, further studies are needed including a prior sample size calculation and defined inclusion criteria (randomisation). It is also necessary to do further studies in other countries, because globally, there are many different managements and thus differences in the IgG supply of the calves.

Conclusions

39.7% of the investigated calves from the regions Enns-Pongau and Lungau, Austria showed Brix values of < 8.4% (cut-off for FTPI). This shows that there is an urgent need for improvement in terms of colostrum management in these specific regions of Austria. This study also determined that the point-of-care tests using whole blood supernatant and plasma carried out between the 3rd and the 6th day of age are suitable to get information on the status of transfer of passive immunity in calves. The point-of-care test showed a sensitivity of 90% and a specificity of 70% for whole blood supernatant and a sensitivity of 84% and a specificity of 72% for plasma using 8.3% Brix as the cut-off. It was feasible to carry out the point-of care test at 12 to 16 h after birth using whole blood collected from the ear edge by the farmer. Further studies are needed to evaluate if an early evaluation of transfer of passive immunity (12 to 16 h *pp*) might be useful and whether the point-of-care test provides an accurate result using whole blood.

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Authors' contributions
CH, KL and TW conceived and designed the study. CH collected samples and analyzed the samples. CH and KL analyzed, interpreted and curated data, wrote the original draft preparation and did the visualization. AT was involved in data analysis and curation. CH, KL, AT and TW validated the data. Formal analysis was done by CH, KL and TW. Funding acquisition and project administration was done by KL, NH and TW. The manuscript was reviewed and edited by CH, KL, NH, AT and TW. KL and TW supervised the project. All authors have read and approved the final version of the manuscript.

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Data Availability
Data available within the article.

Declarations

Competing interests
The authors declare that they have no competing interests.

Consent for publication
Not applicable.

Ethics approval
This study was approved by the Ethics and Animal Welfare Committee (ETK) of the University of Veterinary Medicine, Vienna and the Austrian national authorities, according to § 26 of the Tierversuchsgesetz 2012 – TVG 2012 (GZ.: 2021 – 0.644.875).

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Factors Associated with Colostrum Quality, the Failure of Transfer of Passive Immunity, and the Impact on Calf Health in the First Three Weeks of Life

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Simple Summary: Calves rely on passive immunization with significant quantities of high-quality colostrum within the first few hours of life. If immunoglobulin transfer after birth fails, calves face the failure of transfer of passive immunity. FTPI has been known to lead to high morbidity and mortality rates. Therefore, FTPI constitutes a major animal welfare issue. The objectives of this study were to evaluate the factors associated with colostrum quality and FTPI in calves from dairy farms in Austria and to assess the associations between disease occurrence and FTPI. The number of lactations of the dam and the time lag between parturition and colostrum harvest were significantly associated with low colostrum quality. Colostrum quantity and colostrum quality were factors significantly associated with FTPI. Calf morbidity rates, especially for diarrhea, were significantly associated with FTPI. The present investigation underlines the importance of improving farmers' awareness of colostrum management, especially of the most substantial factors, including colostrum quality and colostrum quantity; it further elucidates the consequences of FTPI for disease occurrence.

Abstract: The objectives of this study were to evaluate factors associated with colostrum quality and FTPI in calves from dairy farms in Austria and to assess the associations between disease occurrence and FTPI in calves. In total, 250 calves and their colostrum samples originating from 11 dairy farms were included in the study. All calves born between September 2021 and September 2022 were included. Blood samples were collected between the third and the sixth day of age. The farmers were trained in disease detection and recorded any health events within the first three weeks of age daily. Multiparous cows (>3 lactation) and colostrum harvesting within the first 2 hours after parturition were significantly associated with good colostrum quality (>22% Brix). Colostrum quantity (≥2 L) and quality (≥22% Brix) acted as protective factors against FTPI (serum Brix ≥ 8.4%) with odds ratios of OR = 0.41 and OR = 0.26, respectively. Calves facing any health event (diarrhea, navel illness, bovine respiratory disease, abnormal behavior) in the first three weeks of life had a higher probability of FTPI. Calves exhibiting diarrhea in the first 3 weeks of life were associated with having FTPI (OR = 2.69). The results confirm the current recommendations for good colostrum management practices and the impact of FTPI on calf morbidity.

Keywords: colostrum quality; poor-quality colostrum; bacterial contamination; FTPI; morbidity; odds ratio; calf diarrhea; navel illness; bovine respiratory disease; health event

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1. Introduction

Calves rely on passive immunity based on immunoglobulins (IgGs) originating from the colostrum, since the cotyledonary synepitheliochorial placenta type of cows acts as a barrier for immunoglobulin transfer during pregnancy [1]. Feeding with sufficient quantities of high-quality colostrum immediately after birth is the most important protective factor in preventing calves from suffering from the failure of transfer of passive immunity (FTPI) [1]. Additionally, the colostrum contains many other essential constituents, for instance, growth factors, hormones, antimicrobial factors, leucocytes, oligosaccharides, mRNA, and nutrients; the actual role of all these constituents is not yet clearly understood [1]. High-quality colostrum is defined as having high immunoglobulin concentrations (≥50 g/L) and low bacterial contamination (total plate counts < 100,000 cfu/mL; coliform counts < 10,000 cfu/mL) [1–3]. Feeding with low-quality colostrum (<50 g/L) is known to be one of the factors significantly associated with FTPI, with an odds ratio of 10.7 (OR = 10.7, 95% confidence interval CI = 4.7–24.2) [4]. The apparent efficiency of immunoglobulin absorption tends to be higher in colostrum with lower levels of bacterial contamination [5]. Additionally, calves fed a second colostrum meal were less likely to suffer from FTPI. Of the 4336 investigated calves, 9.4% of those that received two meals of colostrum experienced FTPI and 22.2% of the calves that received one meal of colostrum experienced FTPI [6]. The definition of FTPI is a serum IgG level below 10 mg/mL, which is equal to serum Brix levels of 8.4% [7–9]. In a meta-analysis of the consequences of FTPI, the adjusted risks (95% CI) for mortality, bovine respiratory disease (BRD), diarrhea, and overall morbidity in the case of FTPI were 2.12 (1.43–3.13), 1.75 (1.50–2.03), 1.51 (1.05–2.17) and 1.91 (1.63–2.24), respectively [10]. A study from Germany noted that calf mortality rates (definition of calf mortality: 2 days to 6 months of age) of above 5% were reported on farms with more than 25% of the calves suffering from FTPI [11]. A study from New Zealand including pasture-based dairy systems also detected greater odds of farmer-recorded animal health events (OR = 1.68) in calves with FTPI [12]. Besides the fact that FTPI leads to significant economic losses due to high morbidity and high mortality, it constitutes a severe animal welfare issue [13]. Studies from different countries show that FTPI occurs frequently, and the number of calves affected with FTPI differs substantially between studies. For instance, 14.1% of the included calves were affected in a study from Scotland (N included farms = 38, N included calves = 370), 27.0% in Germany (2 farms; 216 calves), 33.0% in New Zealand (107 farms; 3819 calves), 43.5% in Switzerland (141 farms; 373 calves), 41.9% in Australia (23 farms; 253 calves), and 19.2% in the USA (413 farms; 2030 calves) [4,14–19]. Low colostrum quality is one of the major risk factors associated with FTPI; it is influenced by multiple factors, which can be partially influenced by the farmer through management. Such management-related factors include the time lag between parturition and colostrum harvest, the presence of the dam during colostrum harvest, colostrum storage procedure, and heat treatment of the colostrum [20–23]. Cow-related factors, such as the number of lactations, genetic parameters, dry period length, ante-partum milk leakage, colostrum quantity, the metabolic status of the cow, and udder health have been noted to influence colostrum quality [4,24–28]. Environmental factors, such as the season of calving and the temperature–humidity index, have been proven to have an impact. There are contradictory results regarding immunoglobulin concentrations, whereas other factors (e.g., management-related and cow-related factors) seem to have stronger effects [26,29].

The objectives of this study were to evaluate the factors associated with colostrum quality and serum Brix values in calves from dairy farms in Austria and to assess whether calf diarrhea, navel illness, and BRD are associated with FTPI within the first three weeks of life.

We hypothesized that (1) multiple factors are associated with a low colostrum quality and (2) a low serum Brix level and (3) that calves experiencing diarrhea, navel illness, and/or BRD have a higher probability of exhibiting FTPI.

2. Materials and Methods

2.1. Ethical Considerations

This study was approved by the Ethics and Animal Welfare Committee (ETK) of the University of Veterinary Medicine, Vienna, and the Austrian national authorities, according to § 26 of the Tierversuchsgesetz 2012—TVG 2012 (GZ.: 2021-0.644.875).

2.2. Farm and Animal Selection

In total, 11 farms in the Austrian federal district of Salzburg volunteered to take part in the study; 250 calves born between 17 September 2021 and 30 September 2022 and their corresponding colostrum samples were included in the study. None of the included farms performed any kind of heat treatment procedure, such as colostrum pasteurization. Additionally, the farms did not use any kind of colostrum replacer.

2.3. Cows

In total, 250 cows were included; of these cows, 213 (85.2%) were healthy throughout the whole dry period and at calving and 11 cows (4.4%) showed a disease before/at parturition. Additionally, 2 (0.8%) of the 250 cows suffered from acute mastitis during the dry period, 1 cow (0.4%) showed retained fetal membranes, 7 (2.8%) suffered from clinical hypocalcemia, and 1 cow suffered from moderate lameness (0.4%). Moreover, 21 cows received a prophylactic treatment with an oral calcium bolus or a Vitamin D_3 injection in combination with oral calcium (N = 8). The cows were grouped as suffering from disease during the dry period or at calving ("disease yes") or not ("disease no"). In total, 15 cows received a vaccination against bovine rota-/corona virus and *E. coli* and 15 cows were vaccinated against a herd-specific salmonella strain. Of the 53 animals (10.0%) showing ante-partum milk leakage, 22 (41.5%) were primiparous cows, 17 (32.1%) received intramammary antibiotics, 4 received an internal teat sealant (ITS) (7.6%), 1 (1.9%) received no medication, and 9 (17.0%) received intramammary antibiotics and an additional ITS, respectively. Ante-partum milk leakage was defined as colostrum leakage during parturition and/or immediately before the first colostrum harvest after calving. Calving assistance was categorized according to three categories: (1) normal calving, i.e., spontaneous delivery with no assistance, (2) assisted calving, with one person assisting at the calving, (3) severe, with >1 person assisting at the calving and/or a vet being consulted.

2.4. Calves

All 250 calves were separated from their mothers within 1 hour of birth. The breeds were Simmental (N = 118; 59.3% female and 40.7% male), Pinzgauer (N = 65; 33.9% female and 66.1% male), and cross-breed calves (N = 44; 45.4% female and 54.6% male). All calves underwent a physical examination on the day of the blood sample collection (3rd to 6th days of age); details of the blood sample collection are summarized in Section 2.6. The time point of the blood sample collection was restricted to 3 to 6 days of age, as described elsewhere (7). The results from the physical examination included a general assessment (general behavior, posture, body condition scoring, hair, skin turgor, and mucus membranes), navel, lung, heart, and abdominal assessments (including auscultation), and a fecal assessment. The detailed assessment methods are summarized in Section 2.7.

2.5. Colostrum Quality Assessment

2.5.1. Colostrum Brix Measurements

Following calving, the colostrum was routinely harvested (via a milking machine or hand milking) by the farmers. Subsequently, the colostrum was collected at the point of calf feeding. For example, for farmers feeding the colostrum using a feeding bucket, the sample was collected directly from the feeding bucket. One sterile 15 mL tube was filled with colostrum with the collector wearing disposable gloves. The colostrum sample was immediately frozen at −20 °C at the farm. All colostrum samples were transported frozen on ice in a polystyrene box to the diagnostic laboratory of the University Clinic for Rumi-

nants, Vienna. In the lab, all samples were thawed in the refrigerator at 5–7 °C and vortexed for 5–10 s (VF2®, IKA®-Werke GmbH & Co. KG, Staufen,, Germany). Subsequently, the Brix refractometer (0 to 85% Brix; HM-DREF-1®, Hebesberger Messtechnik, Neuhofen, Austria) was calibrated using deionized water. Calibration was carried out routinely at the beginning of the analysis and following the measurement of 10 colostrum samples. After calibration, the colostrum was pipetted onto the prism using a 1-way 2 mL plastic pipette. The Brix percentage was recorded. In total, 245 of the 250 colostrum samples could be measured, and 5 samples were excluded due to technical reasons (i.e., error message on the refractometer).

2.5.2. Bacterial Contamination

Bacterial contamination was assessed in the diagnostic laboratory of the University Clinic for Ruminants, Vienna. Bacterial contamination was assessed using cut-off values of 100,000 colony-forming units (cfu) per ml and 10,000 cfu/mL for total plate counts (TPC) and coliform counts, respectively (2). In brief, the colostrum samples were thawed in the refrigerator at 5–7 °C and vortexed for 5–10 s (VF2®, IKA®-Werke GmbH & Co. KG, Staufen, Germany). A 1:10 dilution series using 900 µL of sterile 0.9% physiological sodium chloride solution (B. Braun Melsungen AG, Melsungen, Germany) and 100 µL of native colostrum or the corresponding dilution was prepared in 1.5 mL Eppendorf tubes. Subsequently, 100 µL of native colostrum (1:10) and dilution 1 (1:100) and dilution 2 (1:1000) was pipetted on the Columbia agar plates (containing 5% sheep blood) to assess the TPC. Additionally, 100 µL of the native colostrum (1:10) and dilution 1 (1:100) was plated on MacConkey agar for coliform counts. All agar plates were incubated under aerobic conditions at 37 °C for 18–24 h. Photographs were taken of all agar plates and the colonies were counted using the free available Fiji Software (Fiji®, ImageJ). All visible colonies on the Columbia agar were counted. If the cfu levels were above 300 cfu/plate, the colostrum sample was investigated a second time after an additional dilution step (1:10,000). If the total number of colonies per plate still exceeded 300 cfu/plate; the sample was not further investigated and was considered to be non-assessable ("n.a."). The cfu levels were multiplied with the respective dilution factor and cfu values were given as the cfu per milliliter of colostrum (cfu/mL). This study was conducted alongside another investigation by our group on colostrum management practices in Austria (Hechenberger et al., unpublished).

2.6. Serum and Plasma Brix Measurements

Serum and plasma samples were collected between the 3rd and the 6th day of age by jugular venipuncture using an 18-gauge needle and a Vacutainer system with serum (clot-activator) and EDTA tubes (Vacuette®, Greiner Bio-One International GmbH, Kremsmünster, Austria), respectively. This investigation was performed together with an evaluation of the point-of-care testing used to test for FTPI under field conditions (Hartsleben et al., under review). EDTA and serum tubes were centrifuged at 1500× *g* for 10 min at 20–25 °C at the farm of origin (CGOLDENWALL 800D Electric Centrifuge Medical Lab Centrifuge 4000 rpm with CE 6 × 20 mL, Zhengzhou Jin Chen Electronic Technology Co., Ltd., Zhengzhou, China). Subsequently, the Brix values were determined using a digital Brix refractometer (MA871 Refractometer, Hebesberger, Neuhofen, Austria) under field conditions. FTPI was defined as serum Brix values below 8.4% [7].

2.7. Definition of Calf Diseases

The calves underwent a complete physical examination by a veterinarian before blood sample collection (3rd to 6th days of age, see Section 2.4). A shortened physical examination was carried out by the farmers daily. Before the study, all farmers received individual training from one of the authors (C.H.) on how to perform the shortened physical examination using standard operating procedures (SOPs) and checklists. At least once per day, all calves were examined by the trained farmer. For each calf, an individual "calf card" with checklists was designed and fixed on the box of the respective calf (for details,

see Supplementary Materials Figure S1). These calf cards were created by the authors based on the clinical propaedeutics and the calf cards of the Swiss calf health service (Kälbergesundheitsdienst) [30]. Any pathological findings were noted on the calf card as described on the checklists used by the trained farmer. All diseases were graded as mild, moderate, or severe diseases based on the scoring system described elsewhere [30]. For statistical analysis, the disease scoring was dichotomized from three categories (mild, moderate, severe) to two categories (moderate, severe), whereby "mild disease" was changed to "moderate". Moderate and severe deviations were summarized as "severe".

2.7.1. Calf Diarrhea

Following the SOPs, defecation was assessed by the farmers for type, frequency, and painfulness. Fecal consistency, color, and odor, the degree of digestion, and foreign matter were evaluated. The farmers noted the fecal consistency. The consistency may be categorized as described elsewhere: firm, soft, mushy, liquid, and watery [30] (pp. 147–148). To simplify and unify the findings, the farmers documented the feces as "firm" (=moderate disease), "mushy" (=moderate disease), or "watery" (=severe disease).

2.7.2. Navel Illness

The umbilicus was assessed extra- and intra-abdominally by palpation. During the physical examination on the 3rd to the 6th days of age by one of the authors (C.H.), any sign of inflammation (swelling/changed consistency, heat, pain) and any signs of an umbilical or inguinal hernia or enlarged umbilical arteries/veins were noted [31]. To create a suitable SOP for farmers, only navel thickness (assessed by palpation) was assessed during the shortened daily physical examinations. As described in the clinical propaedeutic, navel thickness was assessed using eight categories [30,31]. For the statistical analysis, the pathological findings were classified as moderate or severe navel illness, defined as two-finger-thick and three-or-more-finger-thick swellings, respectively.

2.7.3. Bovine Respiratory Disease

As described in the SOPs, farmers assessed respiration by counting respiratory rates, coughing, and/or nasal discharge. Physiological respiration was defined as 20 to 40 calm and regular breaths per minute. Pathological changes were classified as follows: fast and shallow breathing (40 to 50 times per minute) was defined as moderate disease, very fast breathing, almost panting, and/or respiratory distress was defined as severe disease [30] (pp. 118–121). Coughing was divided into sporadic coughs (moderate disease) and intermittent coughing, which means more than 3 consecutive coughs (severe disease) [30] (p. 113). The quality of nasal discharge was assessed and categorized as serous discharge (moderate disease) if it had a watery, clear, slightly yellowish and/or greyish appearance. Severe disease was defined as viscous mucous that looked opaque and/or purulent discharge showing a yellow color [30] (p. 90).

2.7.4. Abnormal Behavior

Normal physiological behavior was defined as a bright and alert appearance [30] (p. 54). Pathological findings were classified according to three categories defined as follows: mildly depressed/somnolent (moderate disease), moderately depressed/stupor (severe disease), and severely depressed/coma (severe disease). As per these definitions, mildly depressed calves appear dull, apathetic, and sleepy. When walking, slight swaying is observed, and the eyelids are partially closed. Feed and water intake is reduced. Moderately depressed calves lie down determinedly and show markedly sleepy behavior. They can only be roused by very strong stimuli. Calves with severe depression have delayed reflexes and are recumbent, and their respiration is superficial [30] (p. 55).

2.8. Statistical Analysis

Data were collected and summarized using Microsoft Excel 2016. Subsequently, the data were transferred to IBM® SPSS® Statistics Version 28 (IBM®, New York, NY, USA) for further statistical analysis. Descriptive statistics were carried out and values are shown as the median 25th and 75th percentiles, minimum and maximum, since the Brix values were not normally distributed ($p < 0.05$, Kolmogorov–Smirnov test including Lilliefors correction). The coded values were labeled and factors that might explain an insufficient immunoglobulin concentration in the colostrum and in the calf serum were investigated using a two-step process. The first step was a binary logistic regression and the second step was a multiple logistic regression searching for statistically significant associations ($p < 0.05$). The binary logistic regression was carried out using the dichotomous colostral Brix values (>22%; ≤22%) and serum Brix values (<8.4%; ≥8.4%) as dependent variables. The statistical procedure has been described elsewhere [4]. All factors that might influence the outcome were included as covariates. The binary and multiple logistic regression analyses showed odds ratios (OR) with a 95% confidence interval (95% CI) for the different categories of the covariates compared to the defined reference category. If the OR showed results equal to 1 (OR = 1), there was no association between the factor and the outcome. Factors with odds ratios > 1 (OR > 1) were interpreted as factors that were associated with the outcomes (colostrum quality: >22% or ≤22% Brix; serum brix values: <8.4%; ≥8.4%). Factors with odds ratios < 1 (OR < 1) were interpreted as protective factors. The associations between a low serum Brix value, which was defined as FTPI (Brix < 8.4%), and the occurrence of calf diarrhea, BRD, navel illness, and/or an abnormal general behavior were investigated using disease occurrence (healthy, moderate disease, severe disease) as covariates. Case definitions were used as described in Section 2.7. If the OR showed results equal 1 (OR = 1), there was no association between disease occurrence and the outcome (serum Brix values: <8.4%; ≥8.4%). Factors with odds ratios > 1 (OR > 1) were interpreted as factors that were associated with variable outcomes. Factors with odds ratios < 1 (OR < 1) were interpreted as protective factors. The Hosmer–Lemeshow goodness-of-fit function was applied for all analyses. All implemented tests were interpreted as statistically significant if the p value was <0.05.

3. Results

3.1. Colostrum Quality

Of the investigated colostrum samples, 140 (57.1%) showed poor colostrum quality of ≤22% and 105 (42.9%) a good colostrum quality of >22% (Figure 1). The median colostrum Brix values were 21.2% (min = 7.3%, max = 35.1%, 25th = 18.5%, 75th = 25.0%). Primiparous cows (N = 71) showed median Brix values of 20.9% (min = 7.3%, max = 32.2%, 25th = 16.4%, 75th = 24.2%). Cows in their second (N = 58) and third (N = 49) lactations showed median Brix values of 20.9% (min = 10.9%, max = 28.3%, 25th = 18.0%, 75th = 24.0%) and 21.1% (min = 13.0%, max = 35.1%, 25th = 18.8%, 75th = 23.5%), respectively. All cows with >3 lactations (N = 66) were summarized and processed as one group and yielded median Brix values of 23.8% (min = 11.9%, max = 31.0%, 25th = 20.5%, 75th = 26.2%).

3.1.1. Explanatory Variables for Colostrum Quality

Binary Logistic Regression

Overall, the median number of lactations was 2.0 (min = 1.0, max = 11.0, 25th percentile = 1.0, 75th percentile = 4.0). The median gestation length (N = 234) was 284 days (min = 262, max = 301, 25th = 280, 75th = 288). The median time lag between parturition and the first milking (N = 248) was 75 min (min = 0, max = 960, 25th = 30.0, 75th = 180.0). The median dry period length, excluding primiparous cows (N = 174, missing information = 1), was 8.0 weeks (min = 5, max = 23, 25th = 7.0, 75th = 12.0). The univariable approach showed that the number of lactations, the dry-off procedure, the dry period length, the time to first milking, and ante-partum milk leakage were significantly associated with colostrum quality. The association between coliform counts

and colostrum quality was not evaluated, since all of the 135 investigated colostrum samples were below the threshold of ≤10,000 cfu/mL. Of these, 83 (61.5%) and 52 (38.5%) showed Brix values ≤ 22% and >22%, respectively. The results of the binary logistic regression for colostrum quality are summarized in Table 1.

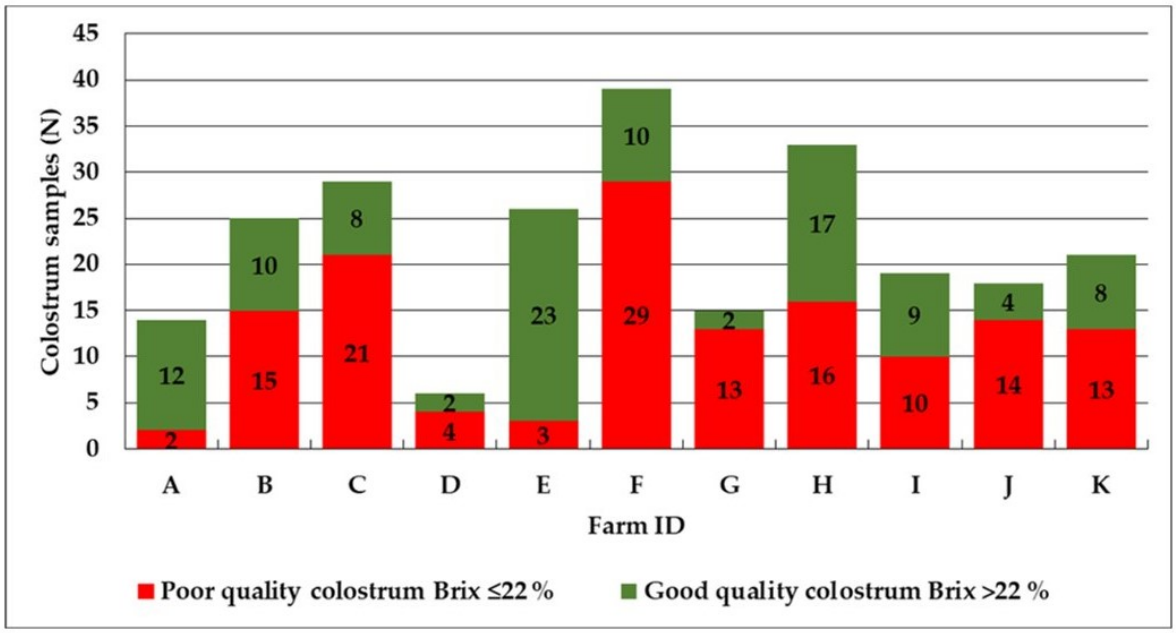

Figure 1. Overview of the 245 colostrum samples originating from 11 dairy farms of the Austrian federal state of Salzburg. The colostrum Brix values were categorized as high-quality colostrum (>22%, green color) and low-quality colostrum (≤22%, red color).

Table 1. Binary logistic regression for factors associated with poor colostrum quality. Poor colostrum quality was defined as a Brix value of ≤22%. Odds ratios (OR) were calculated using the basis category (OR = 1) as a reference. Odds ratios < 1 were interpreted as protective factors for poor colostrum quality and odds rations > 1 were interpreted as factors associated with poor colostrum quality. All statistically significant differences ($p < 0.05$) are highlighted with an asterix * (AB, antibiotic intramammary treatment; ITS, internal teat sealant).

Factor	N Samples Total	Brix ≤ 22%		Brix > 22%		OR (95% CI)
		N Samples	% Samples	N Samples	% Samples	
Number of lactations						
1	71	48	67.6	23	32.4	1
2	58	38	65.5	20	34.5	0.91 (0.44–1.90)
3	49	32	65.3	17	34.7	0.91 (0.42–1.94)
>3	66	22	33.3	44	66.7	0.24 (0.12–0.49) *
Missing	6					
Gestation length						
0–279 days	37	19	51.4	18	48.6	1
>280 days	192	111	57.8	81	42.2	1.30 (0.64–2.63)
Missing	21					

Table 1. *Cont.*

Factor	N Samples Total	Brix ≤ 22%		Brix > 22%		OR (95% CI)
		N Samples	% Samples	N Samples	% Samples	
Ante-partum milk leakage						
Yes	53	37	69.8	16	30.2	1
No	191	102	53.4	89	46.6	0.50 (0.26–0.95) *
Missing	6					
Diseases during dry period						
Yes	11	6	54.5	5	45.5	1
No	234	134	57.3	100	42.7	1.12 (0.33–3.76)
Missing	5					
Time to first milking						
0–119 min	141	65	46.1	76	53.9	1
120–359 min	72	52	72.2	20	27.8	3.04 (1.65–5.61) *
≥360 min	32	23	71.9	9	28.1	2.99 (1.29–6.91) *
Missing	5					
Colostrum harvested						
0–3 L	109	59	54.1	50	45.9	1
4–6 L	104	64	61.5	40	38.5	1.36 (0.79–2.34)
>6 L	29	15	51.7	14	48.3	0.91 (0.40–2.06)
Missing	8					
Total plate count						
TPC < 100,000/mL	100	63	63.0	37	37.0	1
TPC ≥ 100,000/mL	38	23	60.5	15	39.5	0.90 (0.42–1.94)
Missing	112					
Dam vaccination						
Yes	30	22	73.3	8	26.7	1
No	215	118	54.9	97	45.1	0.44 (0.19–1.04)
Missing	5					
Dry-off procedure						
Primiparous cow	69	46	66.7	23	33.3	1
AB	92	60	65.2	32	34.8	0.94 (0.48–1.81)
ITS	37	11	29.7	26	70.3	0.21 (0.09–0.50) *
No medication	11	5	45.5	6	54.5	0.42 (0.11–1.51)
AB+ITS	34	17	50.0	17	50.0	0.5 (0.22–1.16)
Missing	7					
Dry period length						
Primiparous cow	70	48	68.6	22	31.4	1
<8 weeks	59	28	47.5	31	52.5	0.41 (0.20–0.85) *
≥8 weeks	115	64	55.7	51	44.3	0.58 (0.31–1.07)
Missing	6					

Multiple Logistic Regression Colostrum Quality

After the exclusion of the primiparous cows (no information on the dry period length and no dry-off procedure available) and missing values, information on 173 multiparous cows was available. The number of lactations (>3 lactations) turned out to be significantly associated with good colostrum quality (OR = 0.18, 95% CI = 0.04–0.73, p = 0.02). The time lag between parturition and the first milking was statistically associated with poor colostrum quality; for details, see Table 2.

Table 2. Multiple logistic regression for factors associated with poor colostrum quality. Poor colostrum quality was defined as a Brix value of ≤22%. Odds ratios (OR) were calculated using the basis category (OR = 1) as a reference. Odds ratios < 1 were interpreted as protective factors for poor colostrum quality and OR values > 1 were interpreted as factors associated with poor colostrum quality. All statistically significant differences ($p < 0.05$) are highlighted with an asterix * (AB, antibiotic intramammary treatment; ITS, internal teat sealant).

	OR (95% CI)	*p* Value
Number of lactations		
1	1	
2	0.68 (0.17–2.73)	0.59
3	0.71 (0.18–2.85)	0.62
>3	0.17 (0.04–0.70)	0.01 *
Gestation length		
0–279 days	1	
>280 days	1.36 (0.28–6.45)	0.70
Ante-partum milk leakage		
Yes	1	
No	0.70 (0.24–1.99)	0.50
Diseases during the dry period		
Yes	1	
No	0.35 (0.05–2.26)	0.27
Time to first milking		
0–119 min	1	
120–359 min	5.35 (1.83–15.63)	0.00 *
≥360 min	5.43 (1.48–20.00)	0.01 *
Colostrum harvested		
0–3 L	1	
4–6 L	1.18 (0.46–3.04)	0.74
>6 L	0.37 (0.09–1.52)	0.17
Total plate count		
TPC < 100,000/mL	1	
TPC ≥ 100,000/mL	0.57 (0.21–1.50)	0.25
Dam vaccination		
Yes	1	
No	0.58 (0.17–1.98)	0.39

3.2. Physical Examination of the Calves

Milk intake was very good, good, moderate, poor, or absent in 215 (86.0%), 21 (8.4%), 8 (3.2%), 4 (1.6%), and 2 (0.8%) calves, respectively. The median body temperature was 38.9 (min = 38.0 °C, max = 40.5 °C, 25th percentile = 38.7 °C, 75th percentile = 39.1 °C) and the median pulse rate was 117 beats (min = 80, max = 200, 25th = 111, 75th = 124). The detailed results of the physical examinations conducted from the third to the sixth day of age are provided in the Supplementary Materials, Tables S1 and S2.

3.3. Serum and Plasma Brix Values

Of the 250 included calves, 93 (37.2%) and 157 (62.8%) showed an insufficient colostrum supply (FTPI) and a sufficient colostrum supply, respectively (Figure 2). The median serum and plasma Brix values were 8.6% and 9.3%, respectively. The serum and plasma Brix values ranged from 6.3% to 11.8% (25th percentile = 8.1%, 75th percentile = 9.3%) and 7.0% to 12.4% (25th = 8.8%, 75th = 10.1%), respectively.

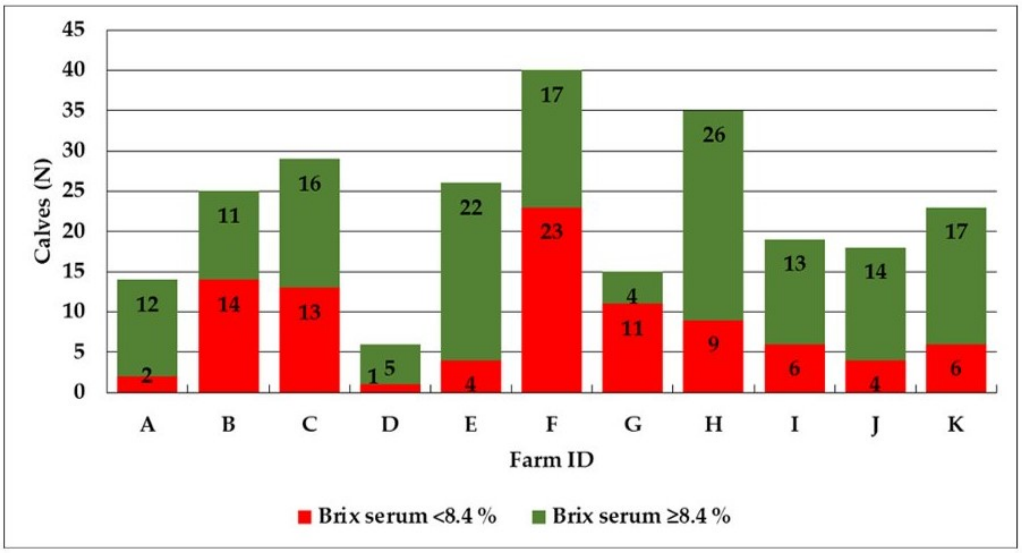

Figure 2. Overview of 250 serum samples from calves originating from 11 dairy farms of the Austrian federal state of Salzburg. The serum Brix values were categorized as a sufficient colostrum supply (≥8.4%, no FTPI, green color) and an insufficient colostrum supply (<8.4%, FTPI, red color), respectively.

3.3.1. Explanatory Variables for Serum Brix Levels

Binary Logistic Regression

The median time lag between parturition and the first feeding (N = 244) was 100 min, (min = 10, max = 1260, 25th percentile = 45, 75th percentile = 195). The median time lag between the first milking and calf feeding was 15 min (min = 0, max = 660.0, 25th = 10.0, 75th = 30.0). The median quantity of colostrum intake was 2.5 L (min = 0, max = 5.0, 25th = 2.0, 75th = 3.0). The colostrum intake of the bottle-fed calves was categorized by the farmers as good in 221 calves and poor in 15 calves. Additionally, 3 calves were fed using an esophageal feeder and 11 calves stayed with their dam. In total, 234 calves received colostrum from their mother and 15 calves from other sources (a cow other than the mother, frozen colostrum stock), (missing value N = 1). The median TPC was 25,850 cfu/mL (min = 0, max = 3,030,000, 25th = 7000, 75th = 121,500). The association between coliform counts and serum Brix levels was not evaluable, since all of the 136 investigated colostrum samples were within the threshold with ≤10,000 cfu/mL. Of these, 57 (41.9%) and 79 (58.1%) showed Brix values < 8.4% and ≥ 8.4%, respectively. The results are summarized in Table 3. The median coliform counts were 0 (min = 0, max = 2150, 25th = 0, 75th = 10).

Table 3. Binary logistic regression for factors associated with low serum Brix levels (< 8.4%) indicating FTPI. Odds ratios (OR) were calculated using the basis category (OR = 1) as a reference. All statistically significant differences ($p < 0.05$) are highlighted with an asterix *. Assisted calving was defined as one person assisting at calving and severe dystocia was defined as more than one person assisting at calving and/or the vet being consulted.

Factor	N Calves Total	Brix < 8.4%		Brix ≥ 8.4%		OR (95% CI)
		N Calves	% Calves	N Calves	% Calves	
Colostrum quality						
≤22% Brix	140	69	49.3	71	50.7	1
>22% Brix	105	21	20.0	84	80.0	0.26 (0.14–0.46) *
Missing	5					

Table 3. *Cont.*

Factor	N Calves Total	Brix < 8.4%		Brix ≥ 8.4%		OR (95% CI)
		N Calves	% Calves	N Calves	% Calves	
Total plate count						
<100,000 cfu/mL	101	44	43.6	57	56.4	1
≥100,000 cfu/mL	38	15	39.5	23	60.5	0.84 (0.40–1.81)
Missing	111					
Time to first feeding						
0–119 min	125	45	36.0	80	64.0	1
120–359 min	92	39	42.4	53	57.6	1.3 (0.75–2.27)
≥360 min	27	6	22.2	21	77.8	0.51 (0.19–1.35)
Missing	6					
Colostrum intake						
<2 L	43	23	53.5	20	46.5	1
≥2 L	190	61	32.1	129	67.9	0.41 (0.21–0.81) *
Missing	17					
Calving						
Unassisted	192	64	33.3	128	66.7	1
Assistance	40	18	45.0	22	55.0	1.64 (0.82–3.27)
Severe dystocia	8	5	62.5	3	37.5	3.33 (0.77–14.39)
Missing	10					

Multiple Logistic Regression

After applying multiple logistic regression, the colostrum quality, time to first feeding, and amount of colostrum fed to the calf showed a significant association with low serum Brix levels, indicating FTPI. For details, see Table 4.

Table 4. Multiple logistic regression for factors associated with low serum Brix values (<8.4%) indicating FTPI. Odds ratios (OR) were calculated using the basis category (OR = 1) as a reference. All statistically significant differences ($p < 0.05$) are highlighted with an asterix *. Assisted calving was defined as one person assisting at calving and severe dystocia was defined as more than one person assisting at calving and/or the vet being consulted.

Factor	OR (95% CI)	*p* Value
Colostrum quality		
≤22% Brix	1	
>22% Brix	0.16 (0.06–0.43)	<0.001 *
Total plate count		
<100,000 cfu/mL	1	
≥100,000 cfu/mL	0.81 (0.33–1.96)	0.64
Time to first feeding		
0–119 min	1	
120–359 min	0.83 (0.33–2.09)	0.69
≥360 min	0.21 (0.06–0.8)	0.02 *
Colostrum intake		
<2 L	1	
≥2 L	0.25 (0.08–0.76)	0.01 *
Calving		
Unassisted	1	
Assistance	2.09 (0.79–5.56)	0.14
Severe dystocia	3.77 (0.62–23.26)	0.15

3.4. Effect of FTPI on Neonatal Diseases

Of the 250 included calves, 45 calves (18.0%), 44 calves (17.6%), and 25 calves (10.0%) experienced a health event of any kind in the first, second, or third week of life, respectively. Initially, health events (any signs of neonatal disease, as defined in Section 2.7) were summarized on a weekly basis and the associations between disease and FTPI were analyzed (for details, see Table 5). The association between disease occurrence and FTPI was the strongest in the third week; for details, see Table 5. Regarding the investigated neonatal diseases, only diarrhea showed a statistically significant association with an odds ratio of 2.69 (95%CI = 1.52–4.76); for details, see Table 6. For BRD, navel illness, the combination of diarrhea and BRD, and altered general behavior, the occurrence was too low for any statistically significant differences to be determined. When calculating the odds ratios for each week (1st, 2nd, and 3rd) for each disease, only diarrhea showed an association with FPTI. In the first week of life, the OR was 2.26 (95%CI = 1.10–4.65), in the second week it was 2.84 (95%CI = 1.38–5.85), and in the third week it was 4.61 (95%CI = 1.81–11.76), including the healthy calves as the basic category (OR = 1). The onset of severe diarrhea in the second and third weeks of life was particularly strongly associated with FTPI with odds ratios of 6.1 (1.6–23.26) and 16.13 (1.98–125.0), respectively. Detailed information on the associations between the occurrence of neonatal diseases and low serum Brix levels are provided in the Supplementary Materials (Tables S3–S8). In the multiple logistic regression, only the occurrence of diarrhea in the first 3 weeks was statistically significantly associated with a low serum Brix level of <8.4% (OR = 3.3, 95%CI = 1.77–6.13, $p < 0.001$).

Table 5. In total, 250 calves were included in the study and any sign of disease, as defined in Section 2.7, was documented for the first three weeks once per day. Signs of diarrhea, navel illness, BRD, and abnormal general behavior, as well as disease combinations, were summarized as "health events" in the binary logistic regression. The odds ratios (OR) were calculated for associations with FTPI (Brix values < 8.4%). Odds ratios were calculated using the basic category = healthy calves (OR = 1) as a reference. All statistically significant differences ($p < 0.05$) are highlighted with an asterix *.

Factor	N Calves Total	Brix < 8.4%		Brix ≥ 8.4%		OR (95% CI)
		N Calves	% Calves	N Calves	% Calves	
First week						
Healthy	205	70	34.1	135	65.9	1
Health event	45	23	51.1	22	48.9	2.02 (1.05–3.87) *
Second week						
Healthy	206	70	34.0	136	66.0	1
Health event	44	23	52.3	21	47.7	2.13 (1.10–4.11) *
Third week						
Healthy	225	76	33.8	149	66.2	1
Health event	25	17	68.0	8	32.0	4.17 (1.72–10.10) *
First to third weeks						
Healthy	168	50	29.8	118	70.2	1
Health event	82	43	52.4	39	47.6	2.60 (1.51–4.49) *

Table 6. Binary logistic regression analysis was carried out using the serum Brix levels as a dependant variable. The table gives an overview of the associations of diarrhea, bovine respiratory disease (BRD), navel illness, diarrhea and BRD, and abnormal general behavior with a low serum Brix level of <8.4% in the first 3 weeks of life. Odds ratios (OR) were calculated using the basic category = healthy calves (OR = 1) as a reference. All statistically significant differences ($p < 0.05$) are highlighted with an asterix *.

Factor	N Calves Total	Brix < 8.4%		Brix ≥ 8.4%		OR (95% CI)
		N Calves	% Calves	N Calves	% Calves	
Diarrhea						
Healthy	182	56	30.8	126	69.2	1

Table 6. *Cont.*

Factor	N Calves Total	Brix < 8.4%		Brix ≥ 8.4%		OR (95% CI)
		N Calves	% Calves	N Calves	% Calves	
Diarrhea	68	37	54.4	31	45.6	2.69 (1.52–4.76) *
BRD						
Healthy	227	86	37.9	141	62.1	1
BRD	23	7	30.4	16	69.6	0.72 (0.28–1.81)
Navel illness						
Healthy	248	92	37.1	156	62.9	1
Navel illness	2	1	50.0	1	50.0	1.69 (0.10–27.78)
Diarrhea and BRD						
Healthy	240	91	37.9	149	62.1	1
Diarrhea + BRD	10	2	20.0	8	20.0	0.41 (0.09–1.97)
Abnormal general behavior						
Healthy	226	81	35.8	145	64.2	1
Abnorm. behavior	24	12	50.0	12	50.0	1.79 (0.77–4.17)

4. Discussion

In total, 250 calves from 11 dairy farms from the federal state of Salzburg (Austria) were included in the study. Of the 245 investigated colostrum samples, 140 (57.1%) showed were of poor quality, with ≤22% Brix. This is similar to the findings from other countries, where poor-quality colostrum has been found in 15.5% to 57.8% [4,14,29] of samples. The aforementioned studies investigated the colostrum samples using radial immunodiffusion (RID). Radial immunodiffusion is currently recognized as the gold standard; elsewhere, it has been noted that cheap and user-friendly indirect measurement methods, such as the digital Brix refractometer, are reliable alternatives for evaluating colostrum and serum immunoglobulin concentrations [15,32,33]. Of the 250 included calves, 93 (37.2%) showed a low serum Brix level of less than 8.4%, indicating FTPI. In plasma, the threshold for FTPI remains higher. An investigation carried out in Germany of the different analytical approaches for assessing FTPI described thresholds of 7.8% for serum and of 8.6% for plasma [15]. The occurrence of FTPI found in this study is comparable to that identified in other investigations, where FTPI was found in between 14.1% and 41.9% of the included colostrum samples [4,14,15,17–19]. There are differences between countries and herds regarding the occurrence of low-quality colostrum and FTPI [19]. In the present study, the best farm had 88.5% high-quality colostrum samples (Farm E) and the worst farm had 13.3% (Farm G) high-quality colostrum samples. Similarly, the number of calves showing a sufficient colostrum supply was 84.6% for the best farm (Farm E) and 26.7% for the worst farm (Farm G). It has been shown that individual farm management factors have a significant impact on FTPI frequency. A study conducted in Quebec (Canada) investigated the herd-level prevalence of FTPI in 59 dairy herds (with a minimum of 14 calves included per herd) and described high herd-level variations in the rates of calves experiencing FTPI, ranging between 30% and 100% [34]. A study conducted in Italy included 21 farms (244 calves) and described a within-herd prevalence of FTPI of 20.0% to 71.4% [35]. This underlines the importance of herd-specific evaluations of colostrum management practices.

In the present study, the variation in colostrum quality was high, with minimum levels of 7.3% and maximum levels of 35.1%. Since good colostrum quality acts as a protective factor for FTPI (OR = 0.26), it is essential to test the colostrum's quality before delivering it to the calf [4]. In an online questionnaire on calf management practices in Austria, only 20.8% of the 1287 included farmers implemented colostrum testing protocols on their farms; of these farmers, 86.1% stated that they perform visual inspection of the colostrum [36]. There is a further need to increase farmers awareness of good colostrum management practices, in which colostrum testing plays an essential role. Another management-related factor proven

to be significantly associated with colostrum quality is the time lag between parturition and the colostrum harvest. Harvesting colostrum 2 to 6 h after parturition showed significantly negative effects on colostrum quality in comparison with colostrum harvested within the first 2 h after parturition (OR = 5.29). Colostrum IgG levels have been described to decrease by 3.7% every hour after parturition [20]. Therefore, it is essential to milk the colostrum as soon as possible after birth. The positive effect of cows having more lactations on colostrum quality has been proven in multiple investigations on colostrum management [29]. In the present study, cows in their second lactation showed the worst colostrum quality, which is in accordance with other studies [4,29,37]. The binary logistic regression showed that ITS acts as a protective factor for poor colostrum quality (OR = 0.21). However, in the present study, this factor was not significant in the multiple logistic regression. It can be hypothesized that this might be due to the reduced probability of milk leakage ante partum and/or during parturition. In the present study, the questionnaire solely considered whether the cow experienced milk leakage during parturition and/or immediately before the colostrum harvest. Further studies are needed to investigate whether ITS might improve colostrum quality. It has been proven elsewhere that bacterial contamination of the colostrum has an effect on the apparent efficiency of immunoglobulin absorption and on the probability of calves experiencing pneumonia [5,38]. Only a few of the investigated colostrum samples showed contamination with coliform bacteria; therefore, it was not possible to calculate the effects on colostrum quality. Additionally, the majority of the TPC values were below the published threshold of <100,000 cfu/mL and the comparative groups were too small [2]. Due to resource issues, it was not feasible to investigate 100% of the colostrum samples for bacterial contamination; further work is needed in this field.

Factors such as feeding the calf with high-quality colostrum (>22% Brix) and higher quantities of colostrum ($\geq$2 L) were revealed to be protective factors for calves facing FTPI, yielding odds ratios of OR = 0.16 and OR = 0.25, respectively. These effects have been proven several times by different research groups and are among the most important factors for ensuring a successful colostrum supply [1,39]. Contradictory results were found regarding the time to first feeding where the time lag (>360 min) seem to be protective (OR = 0.21). In total, 51.2% of the calves were fed within the first 2 hours of life, only 37.7% between 2 and 6 h after parturition, and only 11.1% more than 6 h after parturition. The number of calves in the last group was small and the distribution between calves with and without FTPI was unequal. Therefore, this result must not be over-interpreted. The common recommendations for good colostrum management state that the calf should be fed immediately after parturition [1].

In total, the number of calves showing any signs of BRD, navel illness, diarrhea and BRD, and/or an abnormal behavior was low. Therefore, it was not feasible to assess the association between each disease in the first, second, or third week of life. When summarizing any neonatal disease as a "health event", there was a clear association (OR > 1) between disease occurrence and FTPI. The consequences of FTPI in terms of adjusted risks for diarrhea and BRD were found to be 1.75 and 1.51 in a recent meta-analysis [10]. With the present investigation, we can support the finding that there is a clear association between diarrhea and FTPI, with an odds ratio of 2.69 (1.52–4.76). The findings underline the importance of protecting calves from FTPI to prevent them from contracting neonatal diseases, especially diarrhea. In addition to successful colostrum management, there are multiple management-related, calf-related and environment-related factors that need to be addressed to achieve low on-farm calf morbidity and mortality rates.

In the present study, the farmers showed high levels of commitment and carefully followed the SOPs for physical examinations after receiving detailed training from one of the first authors (C.H.). One of the major limitations of the study was that no sample size calculation could be carried out in advance, since the farms joined the study voluntarily. Therefore, we cannot exclude an over- or under-representation of FTPI. Additionally, the number of calves suffering from pneumonia and navel illness was too low for us to assess the impact of FTPI. Further studies are needed to obtain information on the true prevalence

of FTPI in Austria and to further assess the impact of FTPI on neonatal diseases. Brix refractometry was used as a reference method to indirectly test for immunoglobulin concentrations in colostrum and serum. In future investigations, we recommend implementing the RID, which is currently recognized as the gold standard. In consensus with the literature from Austria, there is potential to improve colostrum management on farms in Austria, which could be addressed by the Animal Health Service. Good colostrum management is an indispensable component of raising healthy youngstock. In Austria, vets and advisers should not just focus on the effective transfer of passive immunity in calves but rather focus on achieving excellent passive transfer in calves and further reducing morbidity and mortality [9].

5. Conclusions

Colostrum harvested from cows within 2 hours of parturition and the colostrum from cows with >3 lactations was of a significantly better quality (>22% Brix). In the present study, the colostrum quality (>22% Brix) and the colostrum quantity (≥2 L) were the most important factors in preventing calves from experiencing FTPI. There is a strong association between the occurrence of calf diarrhea and FTPI within the first three weeks of life. Therefore, a sufficient colostrum supply achieving serum Brix levels greater than 8.4% is essential to protect calves from disease.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ani13111740/s1, Tables S1 and S2 show the results of the clinical assessments of the calves between the third and the sixth days of age by a veterinarian (author C.H.) according to the clinical assessment published by Baumgartner and Wittek, 2018. Tables S3–S8 show the results of the binary logistic regression analysis for the first, second, and third weeks of life using FTPI levels as the dependent variable and disease occurrence as covariates.

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Table S1: Part 1: Overview on the physical examination of 250 calves between the 3rd and the 6th days of age. The physical examination was carried out according to Baumgartner and Wittek 2018.

Clinical assessment	N calves	% calves
General behavior		
Normal	203	81.2
Slightly depressed	43	17.2
Moderately depressed	3	1.2
Severely depressed	1	0.4
Posture		
Normal	227	90.8
Arched back	15	6.0
Extended/sawhorse like	1	0.4
Other	7	2.8
Body condition		
Very good	236	94.4
Good	14	5.6
Hair and skin assessment		
Smooth, shiny, flat lying	229	91.6
Shaggy	19	7.6
Alopecia	1	0.4
Missing	1	0.4
Skin turgor		
Normal	234	93.6
Skin fold remains	1	0.4
Slight enophthalmus	4	1.6
Moderate enophtalmus	1	0.4
Missing	10	4.0
Navel palpation		
Normal	225	90.0
Enlarged	8	3.2
Enlarged and painful	8	3.2
Enlarged, painful and rough	9	3.6
Mucus membranes		
Normal	244	97.6
Anaemia	5	2.0
Cyanosis	1	0.4
Icterus	0	0
Reddened	0	0
Nasal discharge		
No discharge	221	88.4
Serous	27	10.8
Mucous	2	0.8
Pus	0	0

Table S2: Part 2: Overview on the physical examination of 250 calves between the 3rd and the 6th days of age. The physical examination was carried out according to Baumgartner and Wittek 2018.

Clinical assessment	N calves	% calves
Mucus membranes		
Normal	244	97.6
Anaemia	5	2.0
Cyanosis	1	0.4
Nasal discharge		
No discharge	221	88.4
Serous	27	10.8
Mucous	2	0.8
Coughing		
No coughing	226	90.4
Intermitted coughing	12	4.8
Missing	12	4.8
Lung auscultation		
Normal	190	76.0
Moderate aggravated breathing sounds	57	22.8
Focal no respiration sounds	1	0.4
Missing	2	0.8
Heart sounds		
Normal	244	97.6
Heart murmur	6	2.4
Abdominal auscultation		
Intestine contraction normal	224	89.6
Reduced contraction	4	1.6
Increased contraction	22	8.8
Swing and percussion auscultation		
Negative right and left	250	100
Amount of faeces		
+	149	59.6
++	92	36.8
+++	9	3.6
Faecal color		
Grey	1	0.4
Yellow	58	23.2
Ochre	130	52.0
Brown	59	23.6
Reddish	1	0.4
Greenish	1	0.4
Faecal consistency		
Normal	233	93.2
Fluid	9	3.6
Watery	5	2.0
Dry	3	1.2
Faecal admixture		
No	245	98.0
Yes	5	2.0

Table S3: Binary logistic regression analysis was carried out using the serum Brix levels as dependant variable. The table gives an overview on the association of diarrhea, bovine respiratory disease (BRD), navel illness, diarrhea and BRD and an abnormal general behavior with a low serum Brix level in the first week of life. Odds ratios (OR) were calculated using the basis category = healthy calves (OR = 1) as reference.

Factor	N calves total	Brix <8.4 %		Brix ≥8.4 %		OR (95 % CI)
		N calves	% calves	N calves	% calves	
Diarrhea						
Healthy	215	74	34.4	141	65.6	1
Moderate diarrhea	24	13	54.2	11	45.8	2.25 (0.96-5.26)
Severe diarrhea	11	6	54.5	5	45.5	2.29 (0.68-7.75)
BRD						
Healthy	234	87	37.2	147	62.8	1
Moderate pneumonia	9	3	33.3	6	66.7	0.84 (0.21-3.46)
Severe pneumonia	7	3	42.9	4	57.1	1.27 (0.28-5.78)
Navel illness						
Healthy	249	92	36.9	157	63.1	
Moderate enlarged navel	0					n. a.
Severely enlarged navel	1	1	100	0	0	
Diarrhea and BRD						
Healthy	246	92	34.7	154	62.6	
Moderate	2	0	0	2	100	n. a.
Severe	2	1	50.0	1	50.0	
Abnormal general behavior						
Healthy	230	82	35.7	148	64.3	1
Moderate	16	10	62.5	6	37.5	3.05 (1.05-8.55)
Severe	4	1	25.0	3	75.0	0.60 (0.06-5.88)

Table S4: Binary logistic regression analysis was carried out using the serum Brix levels as dependant variable. The table gives an overview on the association of diarrhea, bovine respiratory disease (BRD), navel illness, diarrhea and BRD and an abnormal abnormal general behavior with a low serum Brix level in the first week of life. Odds ratios (OR) were calculated using the basis category = healthy calves (OR = 1) as reference. Moderate and severe disease events were summarized as having the disease (yes) or not having the disease (healthy). All statistically significant values ($p < 0.05$) are highlighted with an asterix *.

Factor	N calves total	Brix <8.4 %		Brix ≥8.4 %		OR (95 % CI)
		N calves	% calves	N calves	% calves	
Diarrhea						
Healthy	215	74	34.4	141	65.6	1
Diarrhea	35	19	54.3	16	45.7	2.26 (1.1-4.65)*
BRD						
Healthy	234	87	37.2	147	62.8	1
Pneumonia	16	6	37.5	10	62.5	1.01 (0.36-2.89)
Navel illness						
Healthy	249	92	36.9	157	63.1	n. a.
Navel illness	1	1	100	0	0	
Diarrhea and BRD						
Healthy	246	92	34.7	154	62.6	1
Diarrhea + Resp. Disease	4	1	25.0	3	75.0	0.56 (0.06-5.44)
Abnormal general behavior						
Healthy	230	82	35.7	148	64.3	1
Abnormal	20	11	55.0	9	45.0	2.21 (0.88-5.56)

Table S5: Binary logistic regression analysis was carried out using the serum Brix levels as dependant variable. The table gives an overview on the association of diarrhea, bovine respiratory disease (BRD), navel illness, diarrhea and BRD and an abnormal general behavior with a low serum Brix level in the second week of life. Odds ratios (OR) were calculated using the basis category = healthy calves (OR = 1) as reference. All statistically significant values ($p < 0.05$) are highlighted with an asterix *.

Factor	N calves total	Brix <8.4 %		Brix ≥8.4 %		OR (95 % CI)
		N calves	% calves	N calves	% calves	
Diarrhea						
Healthy	209	69	33.0	140	67.0	1
Moderate diarrhea	24	12	50.0	12	50.0	2.03 (0.87-4.74)
Severe diarrhea	12	9	75.0	3	25.0	6.1 (1.6-23.26)*
BRD						
Healthy	234	84	37.2	147	62.8	
Moderate pneumonia	4	0	0	4	100.0	n. a.
Severe pneumonia	7	3	42.9	4	57.1	
Navel illness						
Healthy	244	90	36.9	154	63.1	
Moderate enlarged navel	0					n. a.
Severely enlarged navel	1	0	0	1	100.0	
Diarrhea and BRD						
Healthy	240	89	37.1	151	62.9	
Moderate	3	0	0	3	100	n. a.
Severe	2	1	50.0	1	50.0	
Abnormal general behavior						
Healthy	230	83	36.1	147	63.9	
Moderate	14	7	50.0	7	50.0	n. a.
Severe	1	0	0	1	100.0	

Table S6: Binary logistic regression analysis was carried out using the serum Brix levels as dependant variable. The table gives an overview on the association of diarrhea, bovine respiratory disease (BRD), navel illness, diarrhea and BRD and an abnormal general behavior with a low serum Brix level in the second week of life. Odds ratios (OR) were calculated using the basis category = healthy calves (OR = 1) as reference. Moderate and severe disease events were summarized as having the disease (yes) or not having the disease (healthy). All statistically significant values ($p < 0.05$) are highlighted with an asterix*.

Factor	N calves total	Brix <8.4 %		Brix ≥8.4 %		OR (95 % CI)
		N calves	% calves	N calves	% calves	
Diarrhea						
Healthy	209	69	33.0	140	67.0	1
Diarrhea	36	21	58.3	15	41.7	2.84 (1.38-5.85)*
BRD						
Healthy	234	84	37.2	147	62.8	1
Pneumonia	11	3	27.3	8	72.7	0.63 (0.16-2.45)
Navel illness						
Healthy	244	90	36.9	154	63.1	n. a.
Navel illness	1	0	0	1	100.0	
Diarrhea and BRD						
Healthy	240	89	37.1	151	62.9	1
Diarrhea + Resp. Disease	5	1	20.0	4	80.0	0.42 (0.04-3.86)
Abnormal general behavior						
Healthy	230	83	36.1	147	63.9	1
Abnormal	15	7	46.7	8	53.3	1.55 (0.54-4.42)

Table S7: Binary logistic regression analysis was carried out using the serum Brix levels as dependant variable. The table gives an overview on the association of diarrhea, bovine respiratory disease (BRD), navel illness, diarrhea and BRD and an abnormal abnormal general behavior with a low serum Brix level in the third week of life. Odds ratios (OR) were calculated using the basis category = healthy calves (OR = 1) as reference. All statistically significant values ($p < 0.05$) are highlighted with an asterix*.

Factor	N calves total	Brix <8.4 %		Brix ≥8.4 %		OR (95 % CI)
		N calves	% calves	N calves	% calves	
Diarrhea						
Healthy	211	70	33.2	141	66.8	1
Moderate diarrhea	14	8	57.1	6	42.9	2.69 (0.90-8.06)
Severe diarrhea	9	8	88.9	1	11.1	16.13 (1.98-125.0)*
BRD						
Healthy	231	85	36.8	146	63.2	
Moderate pneumonia	2	0	0	2	100.0	n. a.
Severe pneumonia	1	1	100.0	0	0	
Navel illness						
Healthy	233	86	36.9	147	63.1	
Moderate enlarged navel	1	0	0	1	100.0	n. a.
Severely enlarged navel	0	0	0	0	0	
Diarrhea and BRD						
Healthy	231	85	36.8	146	63.2	
Moderate	2	0	0	2	100.0	n. a.
Severe	1	1	100.0	0	0	
Abnormal general behavior						
Healthy	228	82	36.0	146	64.0	
Moderate	6	4	66.7	2	33.3	n. a.
Severe	0	0	0	0	0	

Table S8: Binary logistic regression analysis was carried out using the serum Brix levels as dependant variable. The table gives an overview on the association of diarrhea, bovine respiratory disease (BRD), navel illness, diarrhea and BRD and an abnormal general behavior with a low serum Brix level in the third week of life. Odds ratios (OR) were calculated using the basis category = healthy calves (OR = 1) as reference. Moderate and severe disease events were summarized as having the disease (yes) or not having the disease (healthy).

Factor	N calves total	Brix <8.4 %		Brix ≥8.4 %		OR (95 % CI)
		N calves	% calves	N calves	% calves	
Diarrhea						
Healthy	211	70	33.2	141	66.8	1
Diarrhea	23	16	69.6	7	30.4	4.61 (1.81-11.76)*
BRD						
Healthy	231	85	36.8	146	63.2	1
Pneumonia	3	1	33.3	2	66.7	0.86 (0.08-9.62)
Navel illness						
Healthy	233	86	36.9	147	63.1	n. a.
Navel illness	1	0	0	1	100.0	
Diarrhea and BRD						
Healthy	231	85	36.8	146	63.2	1
Diarrhea + Resp. Disease	3	1	33.3	2	66.7	0.86 (0.08-9.62)
Abnormal general behavior						
Healthy	228	82	36.0	146	64.0	1
Abnormal	6	4	66.7	2	33.3	3.56 (0.64-20.0)

Kälberkarte für das Projekt „Kolostrumversorgung von Kälbern im Bundesland Salzburg und Validierung eines Immunglobulin-Schnelltests“

LFBIS-Nr. Tierhalter: | | | | | | | |

Ohrmarke Kalb: AT __ __ __ __ __ __ __ __ __ __

Tab. 1: Bitte vom 1.-7. Lebenstag ausfüllen

Lebenstag	1			2			3			4			5			6			7		
Art der Tränke[1]																					
Uhrzeit der Tränke																					
Trinkmenge																					
Allgemeinbefinden[2]																					
Kotkonsistenz[3]																					
Atmung[4]																					
Husten[5]																					
Nasenausfluss[6]																					
Nabel[7]																					
Sonstige Erkrankung																					
Besondere Vorkommnisse[8]																					

Tab. 2: Bitte vom 8.-28. Lebenstag ausfüllen

Lebenstag	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Art der Tränke[1]																					
Trinkmenge																					
Allgemeinbefinden[2]																					
Kotkonsistenz[3]																					
Atmung[4]																					
Husten[5]																					
Nasenausfluss[6]																					
Nabel[7]																					
Sonstige Erkrankung																					
Besondere Vorkommnisse[8]																					

Legende auf der Rückseite!

Legende:

1: MAT= Milchaustauscher; V= Vollmilch; S= angesäuerte Milch; K=Kolostrum; T= Transitmilch; J= Joghurttränke; (Sonstige Tränken bitte selbstständig ausfüllen)

2: leeres Feld= ungestört; -=Kalb wirkt müde (ggr. verm.); --=festliegend (mgr. verm.); ---=komatös (hgr. verm.)

3: leeres Feld=normal; B= dünnbreiig; W= wässrig

4: leeres Feld =ungestört; +=Kalb atmet schneller; ++=Kalb atmet wesentlich schneller („hechelt"); +++=Kalb hat Atemnot

5: leeres Feld= ungestört; S=stoßweise (<3 mal); A=anfallsweise (>3 mal);

6: leeres Feld= ungestört; S=serös; M=mukös; E=eitrig

7: leeres Feld= ungestört (ca. 1cm dick); += zweifinger dick; ++= dreifinger dick; +++=handdick

8: Bitte besondere Vorkommnisse, wie zum Beispiel Enthornung, Kastration, Impfung, Umstallung etc. dokumentieren

Ergebnisse einer Umfrage zum Kolostrummanagement im Bundesland Salzburg

Christina Hartsleben[1], Nicole Hechenberger[2], Pia-Desiree Wanke-Jellinek[3], Thomas Wittek[3], Katharina Lichtmannsperger[3]

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[2] Tiergesundheitsdienst Salzburg, Bundesstraße 6, 5071 Wals-Siezenheim
[3] Universitätsklinik für Wiederkäuer, Veterinärmedizinische Universität Wien, Veterinärplatz 1, 1220 Wien

Ziel: Das Kolostrummanagement auf Milchvieh- und Mutterkuhbetrieben im Bundesland Salzburg zu beschreiben.

Hypothesen:
1. Es werden Testgeräte eingesetzt, um die Kolostrumqualität zu erheben.
2. Den Kälbern wird innerhalb der ersten sechs Lebensstunden Kolostrum gefüttert.
3. Die Landwirt:innen legen Wert auf Hygiene bei der Kolostrumgewinnung.

Material und Methode:

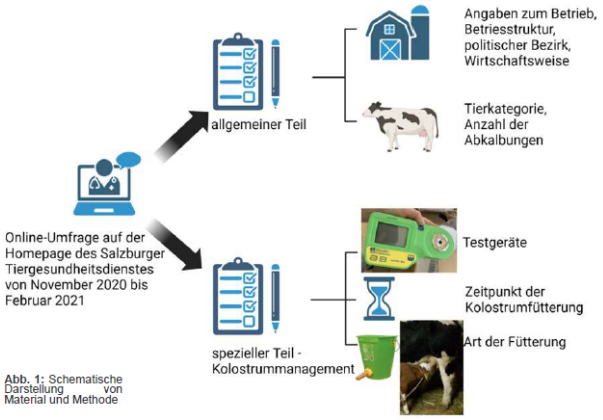

Abb. 1: Schematische Darstellung von Material und Methode

Fragebogen

- 332 teilnehmende Betriebe aus dem Bundesland Salzburg, welche den allgemeinen Teil beantworteten.
- 88 Betriebe davon beantworteten zusätzlich den spezifischen Teil über das Kolostrummanagement.

Allgemeiner Teil des Fragebogens (Abb. 1)

Spezieller Teil des Fragebogens - Kolostrummanagement

- Einsatz von Testgeräten
- Ist eingefrorenes Kolostrum oder Kolostrumersatz am Betrieb gelagert?
- Zeitpunkt und Art der ersten Melkung nach der Geburt
- Zeitpunkt, Art und Darreichungsform der ersten Mahlzeit des Kalbes

Statistische Auswertung

- Microsoft Excel 2010 (Microsoft®, Washington, USA) und IMB® SPSS® Statistics Version 28 (IBM®, New York, USA)

Ergebnisse und Diskussion:

Allgemeiner Teil

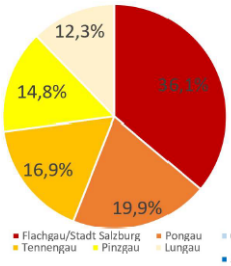

Abb. 2: Verteilung der teilnehmenden Betriebe pro politischer Bezirk.

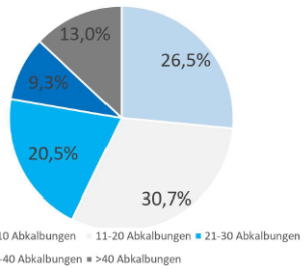

Abb. 3: Kategorisierung nach Anzahl der Abkalbungen.

Spezieller Teil - Kolostrummanagement

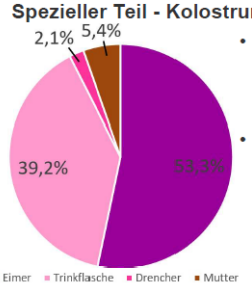

Abb. 5: Art der Verabreichung des Kolostrums.

- Lediglich 6,3% der befragten Landwirt:innen setzen Testgeräte zur Bestimmung der Kolostrumqualität ein
- Kolostrum wird in 56% der Betriebe innerhalb einer Stunde gemolken. 54,5% der Betriebe tränken die Kälber innerhalb der ersten Lebensstunde, 34,9% der befragten Betriebe innerhalb der ersten 6 Lebensstunden

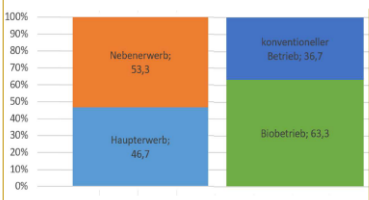

Abb. 4: Überblick über die Verteilung hinsichtlich der Betriebsstrukturen.

Diskussion

- Da lediglich 21 der befragten Betriebe Testgeräte zu Bestimmung der Kolostrumqualität verwenden, besteht hier Verbesserungspotential. Auch in einer vergleichbaren Studie gaben nur 20,8 % der Landwirt:innen an, Testgeräte zu verwenden. (Klein-Jöbstl et al. 2015)
- Auch in Vergleichsstudien werden über 90% der Kälber innerhalb der ersten sechs Lebensstunden mit Kolostrum versorgt. (Klein-Jöbstl et al. 2015; Reschke et al. 2017)
- 89,2 % der Landwirt:innen geben an, dass sie vor der Ermelkung des Kolostrums das Euter reinigen, was den Werten in vergleichbaren Studien entspricht. (Reschke et al. 2017; Hyde et al. 2020)

Conclusio: Durch die Umfrage kann nun gezielt auf die Bedürfnisse der Landwirt:innen hinsichtlich der Beratung zum Thema "Kolostrummanagement" eingegangen werden. Der Einsatz von Testgeräten zur Erhebung der Kolostrumqualität ist verbesserungswürdig. Positiv hervorzuheben ist allerdings, dass ein Großteil der Kälber innerhalb der ersten sechs Lebensstunden Kolostrum erhält und Landwirt:innen diesbezüglich auf Hygiene achten.

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← Hier geht's zu weiteren Ergebnissen unseres Forschungsteams.

← Referenzen

vetmeduni vienna

ÖGT-Tagung in Salzburg, 2023

Zusammenhänge zwischen einer schlechten Immunglobulinversorgung und dem Auftreten von Kälberkrankheiten in den ersten drei Lebenswochen

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[#] Christina Hartsleben und Katharina Lichtmannsperger sind gleichwertig als Erstautoren zu werten.

Abstract: Die Ziele der Studie waren Faktoren, welche mit der Kolostrumqualität und Failure of Passive Transfer of Immunity (FPTI) assoziiert sind zu erheben und die Auswirkung von FPTI auf die Gesundheit der Kälber in den ersten drei Lebenswochen zu evaluieren. 250 Kälber, welche zwischen September 2021 und September 2022 geboren wurden, mit der dazugehörigen Kolostrumprobe nahmen an der Studie teil. Die Tiere stammten aus 11 Betrieben aus der Region Enns-Pongau und Lungau (Salzburg, Österreich). Aus der ersten Mahlzeit, welche dem Kalb angeboten wurde, wurde von den Landwirten eine Probe entnommen und sofort eingefroren. Diese wurde zu einem späteren Zeitpunkt nach Wien transportiert und dort im Labor der Veterinärmedizinischen Universität Wien auf den Brix-Wert (in %) und auf den Keimgehalt untersucht. Die Kälber wurden am dritten bis sechsten Lebenstag klinisch untersucht und Blutproben wurden genommen. Diesbezüglich wurde der Brix-Wert im Serum und im Plasma der Kälber analysiert. Zur Überwachung des Gesundheitsstatus der Kälber wurden die Landwirte in der Erkennung von Krankheiten geschult und notierten täglich alle gesundheitlichen Ereignisse innerhalb der ersten drei Lebenswochen. Kühe mit mehreren Abklabungen (>3 Laktationen) und das Abmelken von Kolostrum innerhalb der ersten zwei Stunden nach der Geburt waren signifikant mit einer guten Kolostrumqualität (>22 % Brix) verbunden. Kolostrummenge (≥2 Liter) und -qualität (≥22 % Brix) wirkten als Schutzfaktoren gegen FPTI (Brix-Wert im Serum des Kalbes <8,4 %) mit Odds Ratios von 0,41 bzw. 0,26. Kälber, bei denen in den ersten drei Lebenswochen ein gesundheitliches Ereignis auftrat, litten häufiger an FPTI. Jene Tiere, die in den ersten drei Lebenswochen Durchfall aufwiesen, waren mit FPTI assoziiert (OR = 2,69). Die Ergebnisse bestätigen die aktuellen Empfehlungen für ein gutes Kolostrummanagement und die Auswirkungen von FPTI auf die Kälbermorbidität.

Korrespondierende Autorin: Mag. med. vet. Christina Hartsleben (christina.hartsleben@yahoo.de)

3. Publikationen/ wissenschaftliche Beiträge als Co-Autorin

Lichtmannsperger K, Hartsleben C, Spöcker M, Hechenberger N, Tichy A, Wittek T. 2023. Factors Associated with Failure of Transfer of Passive Immunity and the Impact on Calf Health [Vortrag]. European Buiatrics Congress and ECBHM Jubilee Symposium, 24. August – 26. August, 2023; Berlin, Deutschland.

Lichtmannsperger K, Hartsleben C, Spöcker M, Tichy A, Hechenberger N, Wittek T. 2023. Factors associated with a Failure of Transfer of Passive Immunity and impact on calf health [Vortrag]. XXII Middle European Buiatrics Congress; 31. Mai – 3. Juni, 2023; Stara Zagora, Bulgaria.

4. Erweiterte Diskussion

In der vorliegenden Arbeit wurde erstmals in Österreich ein Schnelltest zur Überprüfung des Immunglobulin-Status im Blut von Kälbern evaluiert. Zusätzlich wählten die meisten vorliegenden Studien zur Etablierung eines Immunglobulin-Schnelltests einen Zeitpunkt nach den ersten 24 Lebensstunden. In dieser Arbeit wurde der Schnelltest hingegen bereits zwölf bis 16 Stunden nach der Geburt eingesetzt. Der Grund für dieses Studiendesign war, dass bei erfolgreicher Etablierung des Schnelltests in diesem Zeitfenster noch effektiv Maßnahmen vollzogen werden könnten, um FTPI vorzubeugen. Das zweite Zeitfenster zur Schnelltestdurchführung im Rahmen der vorliegenden Untersuchung war vom dritten bis sechsten Lebenstag. In der vorliegenden Arbeit konnte gezeigt werden, dass der Immunglobulin-Schnelltest FASTest® IgG bovine (FASTest® IgG bovine, Megacor, Österreich) eine sehr gute Methode zur Überprüfung von FTPI am dritten bis sechsten Lebenstag der Kälber ist. Der Schnelltest kann einfach, zeiteffizient und kostengünstig direkt am landwirtschaftlichen Betrieb durchgeführt werden. Allerdings konnte nach zwölf bis 16 Lebensstunden mit Vollblut kein zufriedenstellendes Ergebnis erzielt werden. Zu viele falsch positive Ergebnisse lassen die LandwirtInnen fälschlicherweise annehmen, dass die Kälber ausreichend mit Immunglobulinen versorgt sind. In weiteren Studien muss gezeigt werden, ob der gewählte Zeitpunkt im Allgemeinen zu früh zur Erkennung von FTPI ist, oder, ob einzelne Bestandteile des Vollblutes mit der Matrix des Schnelltests interagieren und dadurch die Testlinie des lateral-flow ELISA beeinflusst wird.

Die Anzahl der Kälber, welcher in der vorliegenden Arbeit als Kälber mit FTPI eingestuft wurden, ist mit 39,7 % (90 Kälber) im Vergleich zu anderen Studien eher hoch. Generell schwankt die Zahl der Kälber mit FTPI in der Literatur von 4,75 % (Deelen et al. 2014), 8,3 % (Gamsjäger et al. 2021), 13 % (Zakian et al. 2018), 27 % (Sutter et al. 2020) bis hin zu 43,3 % (Elsohaby et al. 2019). Dies liegt einerseits daran, dass es keine klar definierten allgemein akzeptierten Grenzwerte gibt, sondern die Grenzwerte je nach Messmethode und je nach Studie schwanken. Andererseits gibt es sowohl globale- als auch managementbedingte Unterschiede, deren Auswirkungen auf FTPI noch nicht ganz geklärt sind. Um diese Unterschiede möglich objektiv werten zu können, bedarf es weitere Studien zur Definierung von Grenzbereichen und zur Abklärung, wie stark sich welche umweltbedingten- und managementbedingten Unterschiede auswirken.

Des Weiteren konnte in dieser Arbeit gezeigt werden, wie sich FTPI auf die Gesundheit der Kälber innerhalb der ersten 21 Lebenstage auswirkt. Die Ergebnisse bestätigen, dass Kolostrumqualität und –quantität einen wesentlichen Einfluss auf FTPI haben. Die Erkrankungswahrscheinlichkeit der Kälber, vor allem die Wahrscheinlichkeit an Durchfall zu erkranken (OR = 2,69), hatte einen signifikanten Zusammenhang mit FTPI. Unter den elf teilnehmenden Betrieben gab es hinsichtlich der Prävalenz von FTPI deutliche Unterschiede, welche sich auch in der Erkrankungswahrscheinlichkeit widerspiegelte. Dies zeigt nochmals deutlich, dass das Management eine sehr große Rolle spielt und sehr vielen LandwirtInnen die Bedeutung von Kolostrum bewusst ist. Nichtsdestotrotz bedarf es noch weitere Aufklärung über die essentiellen Punkte des Kolostrummanagements, wie das Testen der Kolostrumqualität. Über dieses einfache Verfahren kann bis zu einem gewissen Maße über die Gesundheit des Kalbes bestimmt werden, denn es zeigte sich, dass Kälber, welche hochwertiges Kolostrum angeboten bekamen auch in den allermeisten Fällen eine optimale Versorgung an Immunglobulinen im Blut aufwiesen. Die landwirtschaftlichen Betriebe waren alle in der Region Enns-Pongau und Lungau. Um eine bessere Aussagekraft hinsichtlich der Prävalenz von FTPI und den damit verbundenen Managementmaßnahmen zu bekommen, müssen daher weitere Studien in ganz Österreich durchgeführt werden.

5. Zusammenfassung

Im Rahmen der vorliegenden Untersuchung wurde der Immunglobulin G-Gehalt im Blut von 250 Kälbern bestimmt, ein qualitativer lateral-flow ELISA validiert und der Gesundheitszustand der Kälber wurde über die ersten 21 Lebenstage verfolgt. Die 250 Kälber stammten aus elf Betriebe im Enns-Pongau und Lungau (Bezirke von Salzburg, Österreich). Von den LandwirtInnen wurde zwölf bis 16 Stunden nach der Geburt der Schnelltest FASTest® IgG bovine (FASTest® IgG bovine, Megacor, Österreich) mit Vollblut, welches aus einer Ohrkapillare gewonnen wurde, durchgeführt. Außerdem froren die LandwirtInnen einen Teil des Kolostrums, welches an das Kalb verfüttert wurde, zur späteren Analyse im Labor ein und notierten den Gesundheitszustand der Kälber über die ersten 21 Lebenstage auf Kälberkarten. Am dritten bis sechsten Lebenstag wurden die Kälber klinisch untersucht und ein Serum- und ein Plasmablutröhrchen wurden abgenommen. Noch direkt am Betrieb wurden Schnelltests mit Vollblutüberstand und Plasma durchgeführt, die Blutröhrchen zentrifugiert und die Brix-Werte sowohl im Serum, als auch im Plasma bestimmt.

Neunzig Kälber (39,7 %) hatten einen Brixwert von < 8,4 % im Serum, welcher als Grenzwert für FTPI genommen wurde. Da zwischen den Betrieben deutliche Unterschiede zu erkennen waren, zeigt dies, dass das Bewusstsein für ein optimales Kolostrummanagement noch gehoben werden muss. Für den FASTest® IgG bovine wurde ein Grenzwert von 8,3 % Brix im Serum ermittelt, wobei die Sensitivitäten bei 90 %, 84 % und 76 %, und die Spezifitäten bei 70 %, 72 % und 79 % für die Durchführung mit Vollblutüberstand, Plasma und Vollblut lagen. Dieser Schnelltest ist somit eine gute Methode, um den Immunglobulin-Status von Kälbern zwischen dem dritten bis sechsten Lebenstag direkt auf dem Betrieb zu testen.

In der vorliegenden Arbeit waren die Kolostrumqualität (> 22 % Brix) und die Kolostrumquantität (≥ 2 l) die wichtigsten Faktoren, um FTPI vorzubeugen. Hinsichtlich der Kälbergesundheit bestand ein signifikanter Zusammenhang zwischen der Erkrankungswahrscheinlichkeit in den ersten drei Lebenswochen und FTPI (OR = 2,60). Vor allem für Kälber, welche an Durchfall litten, konnte ein starker Zusammenhang mit FTPI, mit der OR = 2,69 (1,52-4,76) aufgezeigt werden.

Die Resultate der Arbeit bestätigen die aktuellen Empfehlungen zum Kolostrummanagement und verdeutlichen nochmals den Einfluss und die Konsequenzen von FTPI auf die Gesundheit von Kälbern.

6. Summary

The objectives of this study were to evaluate a calf-side point-of-care test (qualitative lateral-flow ELISA) to detect calves with FTPI and to identify factors associated with colostrum quality, FTPI and their impact on calf health in the first three weeks of life. Two hundred fifty calves from 11 farms in Enns-Pongau and Lungau (districts of Salzburg, Austria) were enrolled in the study. The farmers carried out a calf-side point-of-care test (FASTest® IgG bovine, Megacor, Austria) using whole blood from ear capillary 12 to 16 hours *post partum*. In addition, the farmers collected colostrum aliquots using 15 ml tubes and stored these in the -20°C freezer on the farm for later analysis in the laboratory and recorded the calf's health status on calf cards over the first 21 days of life. Between the 3rd and 6th day of life, the calves were clinically examined and blood samples were taken. The FASTest® IgG bovine was performed on farm using whole blood supernatant and plasma. Furthermore, the blood samples were centrifuged, and Brix values were determined in both, serum and plasma.

Ninety calves (39.7%) had serum Brix values < 8.4%, which was determined as cut-off value for FTPI. Significant differences between the farms show that awareness of optimal colostrum management is still needed to be raised. For the FASTest® IgG bovine a cut-off value of 8.3% Brix in serum with sensitivities of 90%, 84% and 76% and specificities of 70%, 72% and 79% for whole blood supernatant, plasma and whole blood was calculated. This point-of-care test is therefore a good on-farm method to assess the immunoglobulin status of calves between 3rd and 6th day of life.

In the present study, colostrum quality (> 22% Brix) and colostrum quantity (≥ 2 l) were the most important factors in preventing FTPI. With regard to calf health, there was a significant correlation between the probability of an health event in the first three weeks of life and FTPI (OR = 2.60). Especially for calves suffering from diarrhoea, a strong correlation with FTPI could be shown with an OR = 2.69 (1.52-4.76).

The results of the study confirm the current recommendations for colostrum management and once again emphasize the influence and consequences of FTPI on calves' health.

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8. Abbildungsverzeichnis

9. Tabellenverzeichnis

10. Appendix

Probenbegleitschreiben für Einsendung von Biestmilch

im Rahmen des Kolostrum- und Kolostrumversorgung von Kälbern im Bundesland Salzburg-Projektes

Nummer Betrieb (interner Vermerk)
Probe Nummer

Datum Geburt =
Datum Probenentnahme: _____

LFBIS-Nr. Tierhalter: | | | | | | | |

Teil 1: Fragen zur Mutter und zur Geburt

1. **Ohrmarke Kuh:** (AT) __ __ __ __ __ __ __ __ __ __ Name _____
2. **Wievielte Geburt bzw. Laktation?** _____ Laktation
3. **Trockenstehzeit in Wochen:** _____ Wochen
4. **Trächtigkeitsdauer in Tage:** _____ Tage
5. **Wie wurde die Kuh trocken gestellt?**
   - ☐ Antibiotisch
   - ☐ Zitzenversiegler
   - ☐ Keine Medikamente
   - ☐ Sonstiges: _____
6. **Erkrankungen der Kuh in der Trockenstehzeit oder kurz vor/nach der Geburt:**
   - ☐ Ja → **Wenn ja: Welche** Erkrankung und **wann** trat diese auf?

     _____
   - ☐ Nein
   - ☐ Prophylaxemaßnahmen: _____
7. **Hat die Kuh die Biestmilch vor dem Melken laufen lassen?**
   - ☐ Ja
   - ☐ Nein
8. **Wie viel Biestmilch wurde in Summe abgemolken?**
   - ☐ 0 - 3 Liter
   - ☐ 4 - 6 Liter
   - ☐ > 6 Liter
9. **Wurde die Kuh geimpft?**
   - ☐ Ja → **Wenn ja:** Gegen **welche** Erkrankung(en)? _____
   - ☐ Nein

Teil 2: Fragen zum Kalb auf nächster Seite

Teil 2: Fragen zum Kalb

Ohrmarke Kalb: AT __ __ __ __ __ __ __ __ __ __ Zwilling: AT __ __ __ __ __ __ __ __ __ __

Das **Kalb** wurde am _____ um _____ Uhr **geboren**.

Die **Mutter** wurde um _____ Uhr **gemolken**.

Das **Kalb** wurde um _____ Uhr **getränkt**, mit _____ **l** Kolostrum.

1. **Wie hat das Kalb das Kolostrum aufgenommen?**
   - ☐ Kalb trinkt selbstständig
   - ☐ Kalb musste zwangsgetränkt werden mittels _____
   - ☐ Sonstiges: _____
2. **Wurde das Kalb mit dem Kolostrum der eigenen Mutter getränkt?**
   - ☐ Ja
   - ☐ Nein

   Wenn nein, womit: _____
3. **Wurde das Euter vor der Melkung gereinigt?**
   - ☐ Ja
   - ☐ Nein
4. **Geburtsverlauf:**
   - ☐ Ohne Probleme/Spontangeburt
   - ☐ leichte Mithilfe durch den Landwirt/die Landwirtin
   - ☐ schwere Mithilfe durch den Landwirt/die Landwirtin
   - ☐ Hilfe durch den Tierarzt/die Tierärztin
5. **Wo hat die Kuh gekalbt?**
   - ☐ Anbindehaltung: auf ihrem Standplatz
   - ☐ Abkalbebox: ☐ Einzelbox ☐ Gruppenbox
   - ☐ im Laufstall
6. **Hat das Kalb Ergänzungspräparate erhalten?**
   - ☐ orale Selensubstitution
   - ☐ Seleninjektion
   - ☐ Eiseninjektion
   - ☐ Sonstiges: _____

   Wenn ja, wann: _____
7. Sonstige Bemerkungen: _____

Unterschrift des Probennehmers

Begleitschreiben zum Schnelltest FASTest® IgG bovine im Rahmen des Projektes „Kolostrumversorgung von Kälbern im Bundesland Salzburg und Validierung eines Immunglobulin-Schnelltests“

LFBIS-Nr. Tierhalter: | | | | | | | |

Ohrmarke Kalb: AT __ __ __ __ __ __ __ __ __

Zutreffendes bitte ankreuzen X

TEIL 1 (bei jedem Kalb auszufüllen):

1. **Wie viele Stunden nach der Geburt wurde der Schnelltest durchgeführt?**

   ☐ 12 Stunden

   ☐ 13 Stunden

   ☐ 14 Stunden

   ☐ 15 Stunden

   ☐ 16 Stunden

   ☐ Sonstiges: _____

2. **Wie funktionierte die Blutabnahme mittels Einweglanzette?**

   ☐ gut (ausreichend großer Bluttropfen)

   ☐ schlecht (Bluttropfen zu klein oder gar kein Blut)

   ☐ Sonstiges: _____

3. **Erschien eine eindeutige pink-purpurfarbene Kontrolllinie C?**

   ☐ ja

   ☐ nein

4. **Erschien eine eindeutige pink-purpurfarbene Testlinie T?**

   ☐ ja

   ☐ nein

5. **Wie war die Intensität der Kontrolllinie C und der Testlinie T?**

   ☐ C und T waren gleich intensiv

   ☐ C war intensiver als T

   ☐ T war intensiver als C

6. **War außer den zwei Linien (C und T) auch noch etwas zu sehen? (dritte Linie, Bande, ein verschwommenes Feld, etc.)**

---

7. **Wie lange dauerte es, bis das Ergebnis eindeutig abgelesen werden konnte? _____Minuten**

<u>TEIL 2 (einmalig ausfüllen):</u>

1. **Ist die Gebrauchsinformation des Schnelltests verständlich und anwenderfreundlich formuliert?**

   ☐ ja

   ☐ nein → wenn nein, warum? _____

2. **Wie finden Sie die Durchführung des Schnelltests insgesamt?**

   ☐ einfach

   ☐ mittel (erst nach mehrmaligem Durchlesen der Gebrauchsinformation und einigen Fehlversuchen verständlich)

   ☐ schwierig (auch nach mehreren Versuchen nicht verständlich und dadurch sehr zeitaufwändig)

3. **Haben Sie weitere Anmerkungen/Kommentare/Verbesserungsvorschläge?**
