人兽布鲁氏菌病

Brucellosis in Humans and Animals

原　著　MJ　Corbel

主　译　李　晔　田莉莉

副主译　张少先　王宝华

主　审　王大力　范伟兴

审　校　（以姓氏笔画为序）

王　照　李铁锋

余晓花　张海涛

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人兽布鲁氏菌病

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内容提要

布鲁氏菌病是人兽共患传染病，直接或间接接触被感染动物及其制品导致传播。本书原版由世界卫生组织、世界动物卫生组织、联合国粮农组织联合编写，世界卫生组织出版社出版，权威性与科学性高于一般指南和书籍。作者详细介绍布鲁氏菌病流行病学、临床表现、诊断、治疗、预防、监测等，内容全面、具体，科学实用，译文准确、流畅，具有较强的实际指导意义，适合广大疾病预防控制中心从业人员及医学相关科研人员阅读参考。

著译者名单

主要原著者 MJ Corbel

主　译　李　晔　副主任医师，中国疾病预防控制中心鼠疫布鲁氏菌病预防控制基地布鲁氏菌病研究室

田莉莉　医学硕士，中国动物卫生与流行病学中心人兽共患病监测室

副主译　张少先　翻译硕士，中国地方病防治杂志英语编辑

王宝华　主任医师，中国疾病预防控制中心慢病中心

主　审　王大力　主任医师，中国疾病预防控制中心鼠疫布鲁氏菌病预防控制基地

范伟兴　主任医师，中国动物卫生与流行病学中心人兽共患病监测室

审　校（以姓氏笔画为序）

王　照　医学博士，中国疾病预防控制中心鼠疫布鲁氏菌病预防控制基地

李铁锋　主任医师，中国疾病预防控制中心鼠疫布鲁氏菌病预防控制基地

余晓花　医学硕士，中国疾病预防控制中心鼠疫布鲁氏菌病预防控制基地

张海涛　医学硕士，中国疾病预防控制中心鼠疫布鲁氏菌病预防控制基地

中文版序言

布鲁氏菌病是公认的人兽共患病，既影响人的身体健康，又影响畜牧业发展，并可危及食品安全，造成严重的社会和经济损失，已成为全球特别是发展中国家所面临的重要公共卫生问题之一。

2000 年以来中国布鲁氏菌病疫情持续上升，2014 年全国共报告病例 57 222 例，报告发病率 4.22/10 万，在法定传染病排名中从 2000 年的第 16 位上升至 2014 年的第 6 位。目前中国的布鲁氏菌病疫情处于历史上最严重时期，疫情分布在全国 31 个省（区、直辖市）和新疆生产建设兵团，内蒙古、吉林和黑龙江等北方省（区）出现暴发和流行。

为有效应对当前布鲁氏菌病疫情形势，全面提升布鲁氏菌病防治专业人员工作能力，建设一支具有中国特色的布鲁氏菌病防治队伍，亟须一本权威、科学、可行的参考文献，于是这本译著几经波折终于问世了。

本书由世界卫生组织、世界动物卫生组织、联合国粮农组织联合编写、世界卫生组织出版社出版，权威性与科学性高于一般指南和书籍。此书采用中英双语对照的形式，从专业的角度全面阐述了布鲁氏菌病的临床表现、流行病学、诊断、患者治疗、人畜间的预防与控制、监测和部门合作等内容，配以翔实的表格和图例，并在每章后，总结了此章的要点；由浅入深、通俗易懂，能够规范指导我国各级布鲁氏菌病防控人员、临床医生、护士和兽医等开展人畜间布鲁氏菌病预防和治疗工作。

我们在翻译过程中本着尊重原著的原则，逐词逐句、严格推敲，力争与原文保持一致，但由于本文涉及有关布鲁氏菌病的众多领域，加之译者的英文能力、学识水平和经验有限，故译文中难免有不准确甚至错误的地方，故恳请相关专业的同仁给予批评指正，以帮助我们不断提高和完善。

本书出版过程中得到了世界卫生组织出版社、中国人民军医出版社、中国疾病预防控制中心鼠疫布鲁氏菌病预防控制基地和中国动物卫生与流行病学中心的大力支持，在此一并表示衷心的感谢！

译　者

2015 年 7 月

鸣　谢：

感谢各领域专家的杰出贡献以及就本文提出的建设性意见，这些专家熟知在某些地区布鲁氏菌病仍是重要的经济和公共卫生问题；此外，还要感谢瑞士和意大利外交部的经费支持。

——MJ Corbel, SS Elberg and O Cosivi（编者）

本书缩写词

2-ME	2-巯乙醇
BCV	布鲁氏菌化学疫苗
CF	补体结合
CFT	补体结合试验
CIEP	对流免疫电泳
CNS	中枢神经系统
CSF	脑脊液
CT	X线计算机断层扫描成像
DNA	脱氧核糖核酸
DTT	二硫基苏糖醇
ELISA	酶联免疫吸附试验
U	国际单位
LPS	脂多糖
MRT	乳环试验
NMR	磁共振
PCR	聚合酶链反应
RBT	虎红平板试验
RES	网状内皮系统
SAT	试管凝集试验
S-LPS	光滑脂多糖
SMZ	磺胺甲噁唑
TMP	甲氧苄啶

目　录

1. 简介

布鲁氏菌病又称波状热、地中海热或马耳他热，是一种人兽共患传染病，所有人均可被感染，无年龄、性别差异，通过直接或间接接触被感染动物或其制品传播。尽管在许多国家布鲁氏菌病防控取得了很大的进展，但许多疫源地仍存在畜间的持续传播，从而频繁传播给人。布鲁氏菌病在世界上许多地区是重要的人间传染病，尤其是欧洲的地中海国家、北非、东非、中东、南亚、中亚、中美、南美[1]，然而该病鲜为人知，很少被报道。在少数无官方病例报道的国家，去过该病流行地区的人群中也有病例出现。

布鲁氏菌病是人兽共患病，直接或间接接触被感染动物及其制品而传播给人。尽管许多国家布鲁氏菌病防控取得了巨大进展，但许多疫源地仍存在畜间的持续传播，从而频繁传播给人。

城镇化和畜牧业的发展，加之动物饲养和食品加工缺乏防控措施，导致布鲁氏菌病传播，这给公共卫生带来了一定程度的风险。由于国际旅游的发展，刺激了可能被污染的异国奶制品的消费，例如鲜奶酪等类似食品被带到无布鲁氏菌地区，这也让人们日益关注布鲁氏菌病。

布鲁氏菌病较长的病程使病人长时间不能正常活动，这不仅是一个经济问题，也是一个重要的医疗问题。及时诊断和抗生素治疗可极大地减少病人的失能时间，但许多地区缺乏有效的诊断或治疗措施，还有些地区没有完全执行人兽布鲁氏菌病的检测和预防项目。在这些地区，动物疾病仍然威胁人类健康，尤其是那些社会底层人群。

布鲁氏菌病可以隐性感染，也可以非典型发病。许多患者因症状温和而被误诊，事实上应注意的是，即便严重感染仍很难进行鉴别诊断。谨慎的实验室操作及其结果的解读，有助于对布鲁氏菌病进行鉴别诊断。

当某些地区引进更先进的技术时，不能忽略控制反刍动物布鲁氏菌病的基本科学信息和方法。即使在动物布鲁氏菌病疫情未被控制地区，也应采取相应的方法去预防感染和治疗患者。

初级卫生保健工作中，部门合作在控制布鲁氏菌病方面发挥了重要作用，并为畜牧生产、食品卫生、卫生保健领域的基础设施建设做出了巨大贡献。换句话说，布鲁氏菌病的防控需要得到食品安全与健康教育等多部门的支持。

本文重点阐述环境和职业卫生的基本措施在社区和家庭中的应用以及对患者的检测和治疗。

[1] 世界动物卫生组织资料，包括人间和畜间布鲁氏菌病，都可以在全球动物疫病信息查询库（Handistatus） Ⅱ或“世界动物卫生”的出版物中查询。信息来源于世界动物卫生组织中央调查局在法国巴黎对兽医局（OIE）、联合国粮农组织（FAO）、世界卫生组织（WHO）的联合调查问卷，可以访问下面的网站：http://www.oie.int

2. 临床表现

布鲁氏菌病本质上是动物性疾病，特别是家畜疾病，人因为感染布鲁氏菌而成为意外宿主。换句话说，布鲁氏菌病是人兽共患病。基于遗传证据，布鲁氏菌属一直被认为是羊种布鲁氏菌的变种，为了满足实际需要，将布鲁氏菌属分为 6 个种：牛种、猪种、羊种、沙林鼠种、绵羊附睾种和犬种。从海洋哺乳动物中至少分离到 3 组菌株，并被认定为新的布鲁氏菌种。

布鲁氏菌“种”的区分受到微生物种类及其来源的影响，在流行病学方面有着重要意义，但对人患病程度上影响较小。牛种布鲁氏菌正常情况下与牛有关，羊种布鲁氏菌与绵羊和山羊有关，猪种布鲁氏菌与猪有关（其中猪 4 与猪 5 型分别与驯鹿和啮齿类动物有关），绵羊附睾种感染绵羊而不感染人，猪 5 型菌仅在少数啮齿动物中被分离出来，犬种通常与狗有关，偶尔也感染人，沙林鼠种较少被分离到，且从未发现与人有关。

人感染布鲁氏菌病通常表现为急性发热性疾病，可持续并进展为有严重并发症的慢性失能性疾病。人的感染直接或间接来自于动物传染源，其中牛、绵羊、山羊、猪及其副产品是迄今为止最重要的传染源。这些自然宿主间，主要通过患畜生殖道传播，通常导致流产。人感染通常来源于生殖器排泄物和奶制品。

人或动物感染布鲁氏菌病的临床表现无特异性，诊断需要实验室检测来支持。对于人布鲁氏菌病，可以进行有效的治疗，但是通过控制动物感染、加强个人卫生和提高公共卫生水平等措施进行预防才是最理想的。

2.1 人布鲁氏菌病

布鲁氏菌病是急性或亚急性发热性疾病，常见热型为间歇热或弛张热，伴随不适、厌食和乏力，在无特异性治疗的情况下，可持续数周或数月。除肝、脾和（或）淋巴结肿大之外，其他器官系统几乎不出现显著的体征。急性期可发展成慢性并伴有复发性感染、持续的局部感染或类似于疲劳综合征的非特异性综合征。布鲁氏菌病是由布鲁氏菌属细菌感染引起，诊断该病必须靠细菌分离或特异性抗体检测。

诊断依据包括：

- 已知近期暴露史或布鲁氏菌来源，包括常见的宿主，尤其是牛、绵羊、山羊、猪、骆驼、牦牛、水牛或狗；食用生的或未煮熟的奶或奶制品以及动物的肉和内脏会导致感染。此外，微生物抵抗力及其高传染性使环境污染成为可能的危险之一，尽管这很难被证实。职业暴露和（或）居住在流行区，也能提高诊断概率。
- 从患者体内分离到布鲁氏菌。

- 聚合酶链反应（PCR）证实血液或其他组织样本中存在布鲁氏菌遗传物质。
- 血清学方法证实血液或其他组织样本中存在布鲁氏菌抗原。
- 无交叉抗原暴露时，布鲁氏菌病血清学检测抗体滴度上升。
- 经补体结合试验或标准抗原的 ELISA 试验证实，IgG 抗体滴度维持高位。

人布鲁氏菌病的易感性取决于多种因素，包括免疫状态、感染途径、染菌数量，某种程度上也取决于布鲁氏菌的“种”。尽管严重的并发症可出现在任何布鲁氏菌种的感染中，但是一般来说，羊种和猪种比牛种和犬种对人毒力更强。

常见感染途径包括经皮肤切口或擦伤直接感染、经眼结膜感染、吸入有传染性的气溶胶、食用未经消毒的奶及奶制品。输血、器官移植和性传播是罕见的传播途径。

布鲁氏菌病在经过 2 ～ 3 周的潜伏期后，约 1/2 的病例表现为急性发病，另 1/2 则发病较为隐蔽，要数周或数个月后才出现症状和体征。临床表现多样且非特异，包括发热、出汗、乏力、萎靡、厌食、消瘦、头痛、关节痛和背痛。通常患者早晨感觉较好，症状随着时间推移而加重，疲乏且普遍精神不振。如果不经治疗，热型为间隔数天的上升与下降（“波状热”）。见表 1。

表 1　500 例感染羊种布鲁氏菌的病例症状和体征

症状和体征	病例数	%
乏力	473	95
发热	464	93
疼痛	457	91
出汗	437	87
关节或背痛	431	86
寒战	410	82
头痛	403	81
食欲缺乏	388	78
消瘦	326	65
脊柱压痛	241	48
便秘	234	47
腹痛	225	45
关节炎	202	40
失眠	185	37
淋巴结肿大	160	32
精神状态不佳	127	25
脾大	125	25
咳嗽	122	24
面色苍白	110	22
睾丸痛 / 附睾炎	62	21 [(1)]
肝大	97	19
皮疹	72	14

（续　表）

症状和体征	病例数	%
腹泻	34	7
中枢神经系统异常	20	4
心脏杂音	17	3
肺炎	7	1
黄疸	6	1

引自：MM Madkour. 布鲁氏菌病综述．Madkour's Brucellosis，2 版．柏林：斯普林格

[(1)] 290 例男性中

布鲁氏菌属是兼性细胞内寄生菌，能在宿主的吞噬细胞内生存并繁殖。布鲁氏菌躲避细胞杀灭的机制尚不完全清楚，然而，布鲁氏菌最终被隔离在网状内皮系统的单核细胞和巨噬细胞内，如淋巴结、肝、脾和骨髓。布鲁氏菌可感染全身的任何器官和组织。当临床症状与某特定器官有关时，被称为“局限性”，通常局限在网状内皮系统的器官内。

尽管体液抗体在抗感染过程中起到了一些作用，但布鲁氏菌病康复的首要机制是由细胞介导的免疫。细胞免疫包括特定细胞毒性 T 细胞的发育和巨噬细胞的激活，增强他们的杀菌活性，靶向辅助 T 淋巴细胞释放细胞因子（如 γ 干扰素和肿瘤坏死因子）。细胞介导免疫的同时，宿主通常表现为对布鲁氏菌的皮肤迟发型超敏反应。

2.1.1　骨关节并发症

骨和关节病变是布鲁氏菌病最常见的并发症，40% 以上病例可出现。包括骶髂关节炎、脊椎炎、外周关节炎、膝关节炎、滑囊炎和腱鞘炎等在内的各种综合征均有报道。布鲁氏菌骶髂关节炎尤其常见。患者出现发热和背痛，经常辐射到下肢（坐骨神经）。特殊情况下，儿童拒绝走路和负重。发病初期的 X 线和骨扫描结果可为正常，及时进行 X 线断层扫描（CT）和磁共振成像（NMR）可显示椎间盘变窄。通过放射性核素成像显示脊椎椎体损坏，即脊椎骨髓炎。腰椎比胸椎、颈椎更易受到侵犯。布鲁氏菌病与脊柱结核相比，通常较少造成椎旁脓肿。感染后的脊椎关节病变被认为是由循环免疫复合物引起。

2.1.2　胃肠道并发症

布鲁氏菌病（尤其羊种菌感染时）经常由未经消毒的奶及奶制品等食物传播导致，例如奶酪是常见的传播载体。食物传播布鲁氏菌病与伤寒类似，系统症状多于胃肠道症状，但一些患者也会出现恶心、呕吐和腹部不适。回肠炎、结肠炎、自发细菌性腹膜炎罕有报道。

2.1.3　肝胆并发症

布鲁氏菌病常累及肝，尽管肝功能检测指标正常或略有上升。肝的组织学变化多样，但牛种菌感染可呈现与结节病变不能区分的上皮样肉芽肿。羊种菌感染的病例肝损伤谱已被描绘出来，包括类似病毒性肝炎的炎性分散小结节，在肝细胞坏死区的肝实质内可发现大的

炎性细胞聚合物。在某些病例中也能发现小的散在的上皮样巨细胞肉芽肿。

尽管肝脏受到累及，但肝坏死后的硬化是极其少见的。猪种菌感染可出现肝脓肿、肝或其他组织的慢性化脓性病变。布鲁氏菌病引发的急、慢性胆囊炎也有报道。

2.1.4 呼吸道并发症

吸入传播是公认的布鲁氏菌病传播途径，尤其在屠宰感染动物的屠宰场。肺门（管）旁淋巴结肿大、间质性肺炎、支气管肺炎、肺囊肿、胸腔积液、脓胸等多种肺部并发症均有报道。极少从血痰中分离到布鲁氏菌。

2.1.5 泌尿生殖系统并发症

睾丸炎和附睾炎是男性布鲁氏菌病最常见的泌尿生殖系统并发症，常发生在单侧，布鲁氏菌睾丸炎与睾丸癌或睾丸结核相似。尽管在人类精子库中已发现布鲁氏菌，但经性传播布鲁氏菌病鲜有报道。布鲁氏菌病极少累及肾，但它也可导致类似肾结核的症状发生。女性患者的盆腔脓肿和输卵管炎罕有报道。

2.1.6 妊娠与哺乳

布鲁氏菌病妊娠期间有自然流产和宫内传播的风险，流产是动物布鲁氏菌病常见的并发症。定位在胎盘被认为与赤藓醇（牛种菌生长刺激剂）有关。尽管赤藓醇不出现在人的胎盘组织，但布鲁氏菌菌血症可导致人流产，尤其在妊娠早期。尚不清楚布鲁氏菌导致的流产率是否高于其他细菌，妊娠期间布鲁氏菌病的早期诊断和治疗可以挽救胎儿的生命。

通过乳汁传播这种人传染人的报道极其罕见。

2.1.7 心血管系统并发症

感染性心内膜炎是最常见的心血管系统表现，也是最常见的布鲁氏菌病致死原因。约 2% 的患者有心内膜炎报道，可累及自体瓣膜和人工瓣膜，且主动脉瓣比二尖瓣更易受累。当感染猪种布鲁氏菌时，主动脉窦和其他血管结构的动脉瘤更多见，细菌性动脉瘤作为感染性心内膜炎的神经系统并发症，通常会累及大脑中动脉。布鲁氏菌引起的感染性心内膜炎的治疗通常需要联合应用抗生素和心脏瓣膜置换手术。

2.1.8 神经系统并发症

神经型布鲁氏菌病是指与布鲁氏菌病有关的多种神经系统并发症。约 5% 的羊种布鲁氏菌感染病例出现中枢神经系统受到侵犯。脑膜炎和脑膜脑炎是最常见的临床表现。布鲁氏菌脑膜炎可以是急性的也可以是慢性的，经常在病程后期发生，但也可以作为首发表现。脑脊液分析通常提示蛋白含量升高，葡萄糖浓度正常或偏低，并且脑脊液淋巴细胞增多。事实上我们很少能从脑脊液中分离到布鲁氏菌，但可在脑脊液和血清中检测到特异性抗体。布鲁氏菌病引起的其他中枢神经系统表现包括脑血管炎、细菌性动脉瘤、大脑和硬膜外脓肿、梗死、出血、小脑共济失调。周围神经系统并发症包括神经（神经根）病变、格林－巴利综合征、脊髓灰质炎样综合征。

脑膜炎的脑扫描（如 CT，MRI）通常正常，但可用其检测占位性病变和硬膜外腔的完整性。有报道神经型布鲁氏菌病患者可出现基底神经节钙化。

2.1.9 皮肤并发症

布鲁氏菌病可造成多种皮肤损害，包括皮疹、结节、丘疹、结节性红斑、瘀点、紫癜。皮肤溃疡、脓肿、化脓性淋巴管炎则常出现于猪种布鲁氏菌感染。偶尔出现鼻出血、牙龈出血、血尿、皮肤紫癜，这是由于脾功能亢进、骨髓吞噬作用和（或）抗血小板抗体导致血小板急剧减少。

2.1.10 眼部并发症

布鲁氏菌病引发的眼部并发症虽然少见，但也可造成多种眼部损害。葡萄膜炎是最易发生的眼部并发症，可出现慢性虹膜睫状体炎、钱币状角膜炎、多灶性脉络膜炎和视神经炎。因未曾从人眼部结构中分离到布鲁氏菌，故这些损伤被认为是晚期并发症，可能由免疫介导。因此，通常用糖皮质激素治疗眼部并发症。

2.1.11 慢性布鲁氏菌病

慢性布鲁氏菌病的诊断由于缺乏一条公认的定义而富有争议。多数官方认同将临床症状持续 12 个月及以上（距离诊断时间）的患者称为慢性布鲁氏菌病患者。根据该标准，患者被分为三类：①复发；②慢性局部感染；③延迟康复。

复发是指疗程结束后一段时间再次出现典型的症状和体征（伴或不伴细菌培养阳性）。典型的复发病例有客观的感染体征，如发热、血清 IgG 抗体滴度持续升高。大部分复发出现在停止治疗后 6 个月内，虽然在利福平或链霉素单一用药后曾出现过复发，但通常不是因为抗生素耐药菌株的出现，所以可以用相同药物的重复疗程来治疗复发。

慢性局部感染是指由于骨髓炎、深部组织脓肿等深部感染灶清除失败而导致典型的症状和体征再次出现（伴或不伴细菌培养阳性）。局部感染病例有发热等客观的感染体征，然而在很长一段时期内，症状可以间歇性复现。这种复发病例的局部感染通常以血清 IgG 抗体持续升高为特征。不同于复发，慢性局限性布鲁氏菌病除进行抗生素治疗外，可能需要外科手术清除感染灶。

延迟康复是指有持续的症状但无发热等客观的感染体征。疗程结束的病例抗体滴度下降，甚至消失。延迟康复的病因不明，一些病例的心理学研究显示，在布鲁氏菌病发病之前这些患者人格障碍的发生率较高。重复抗生素治疗于延迟康复病例无益。

2.1.12 儿童布鲁氏菌病

儿童布鲁氏菌病曾被视为罕见，现在认为布鲁氏菌可以感染全年龄组人群，尤其在羊种菌流行地区。无论病例年龄大小，感染进程和并发症发生率都是相似的。

人布鲁氏菌病要点

- 人布鲁氏菌病通常表现为急性发热性疾病。
- 羊种布鲁氏菌感染人群最多。
- 所有年龄组均可感染。
- 并发症可以影响任何器官系统。
- 疾病可存在复发、慢性局部感染或延迟康复。

2.2 动物布鲁氏菌病

布鲁氏菌病是侵袭多种动物的亚急性或慢性疾病。在牛、绵羊、山羊等反刍动物和猪中，感染早期常不明显。成年动物感染集中在生殖系统，通常产生胎盘炎，随后母畜在怀孕后期流产，而雄性牲畜可有睾丸炎和附睾炎（彩图 1 和彩图 2）。单靠临床症状不能确诊，当分离到布鲁氏菌、检测到抗原或遗传物质、检测到特异性抗体、证明存在细胞介导的免疫反应其中之一时方可确诊。

许多动物均可患布鲁氏菌病，尤其是家畜，如绵羊（特别是奶羊）、山羊、牛、猪，一些地区的骆驼、水牛、牦牛、驯鹿也易患病。目前公认的 6 种布鲁氏菌，有 5 种可在一种或多种动物宿主中引起感染和临床症状（表 2），有 4 种可对人类致病：羊种、猪种、牛种和犬种，其致病性递减。最近公认与海洋动物有关的种型对人也有致病力。

表 2　布鲁氏菌属感染的动物

宿主	牛种	羊种	猪种	犬种	绵羊附睾种
牛	+	+	+（罕见）	–	–
水牛	+	+	–	–	–
北美野牛	+	–	–	–	–
绵羊	+（罕见）	+	+（可能）	–	+
山羊	+（罕见）	+	–	–	–
猪	+（罕见）	+（罕见）	+	–	–
狗	+	+	+（罕见）	+	–
骆驼	+（罕见）	+	–	–	–
北美驯鹿 / 驯鹿	–	–	+（4 型）	–	–
麋鹿	+	–	–	–	–
马	+	+（罕见）	+（罕见）	–	–
啮齿类动物	+（罕见）	+（罕见）	+（5 型）	–	–

布鲁氏菌略有宿主专一性，但跨物种感染也可发生，尤其是羊种布鲁氏菌。许多野生动物的感染曾被报道，除明显影响其种群繁殖力外，感染人十分罕见。然而，羊种菌感染奶牛群则会对经济和公共卫生造成严重影响。

被感染的绵羊和山羊具有高度传染性，原因有：羊种菌的致病性、同群羊或牛的近距离接触、混群和圈舍中的重度暴露。动物间的传播是由环境中大量的布鲁氏菌播散导致。

人被感染通常由于直接接触动物或食用被污染的奶制品。人间病例的出现提示可能存在畜间疫情，并可能是畜间疫情监测信息的唯一来源。然而，确定传染源始发本地还是外地十分重要，如果感染原因涉及食品因素，更要确定这类食品为当地生产还是从外地引入。

动物感染布鲁氏菌后症状多不典型，可有流产、早产和胎盘滞留等。一些地区流产相对少见。非洲某些地区，感染牛种 3 型布鲁氏菌的游牧或半游牧畜群主要的临床症状是水囊瘤和脓肿。早产会导致产奶量下降。影响生育通常是暂时的，大部分被感染动物只流产一次或不受影响。动物乳房可持续感染，特别是奶牛和山羊，因此鲜奶也常被布鲁氏菌污染。羊被羊种菌和绵羊附睾种菌感染后，造成的局部感染可导致睾丸炎和附睾炎。山羊、牛、猪、犬分别被羊种菌、牛种菌、猪种菌、犬种菌感染后，可有相似并发症。被羊种菌感染的绵羊和山羊极少发生关节炎。马被布鲁氏菌感染后滑囊局部脓肿可能是其唯一症状。在某些国家中，骆驼奶被羊种菌污染是严重的公共卫生问题。骆驼患布鲁氏菌病后极少出现临床症状。

疾病的严重程度取决于许多因素，例如前期免疫、畜龄、性别和管理（如畜群大小和密度）。流产在未免疫的动物中更为普遍，造成病原体播散的数量也更大。在胎盘、胎膜、羊水、乳房和淋巴结周围的组织和体液中可找到布鲁氏菌。

大部分感染是由于通过患病动物或受污染的饲料而摄入布鲁氏菌，然而，通过呼吸道暴露或擦伤的皮肤黏膜也会引起感染。猪和犬的自然繁殖可导致传播，绵羊和山羊次之。在猪和犬中普遍存在持续的菌血症，其他动物的感染期间通常出现间歇或短暂的菌血症。

动物布鲁氏菌病要点

- 布鲁氏菌可感染多种动物，尤其是牛、绵羊、山羊和猪。
- 不同种型的布鲁氏菌优先感染的动物不同。
- 动物布鲁氏菌病典型表现是流产。
- 只有通过实验室检测方可确诊。

3．流行病学

3.1 人布鲁氏菌病流行病学

3.1.1 传染源

布鲁氏菌病是动物传染病，因此，最终传染源是被感染的动物。主要传染源是牛、绵羊、山羊、猪等重要的家畜，野牛、水牛、骆驼、犬、马、驯鹿和牦牛是次要传染源，但在某些地区它们也可能是非常重要的传染源。最近发现海豚（含无喙海豚）、海豹等海洋哺乳动物也可成为传染源，职业人群接触其受感染组织后，存在被感染的风险。

患病风险及严重程度很大程度上取决于所暴露的布鲁氏菌种型，其种型会受到作为传染源的动物宿主种类的影响。

人间布鲁氏菌病最频繁被报道和最常被分离到的是羊种布鲁氏菌。它是毒力最强的种型，与严重急性疾病有关。某些国家将占很大比例的羊种菌引起的人布鲁氏菌病作为地方病。羊种菌通常感染绵羊和山羊，也可感染犬、牛、骆驼等。在某些国家，特别是中东国家，牛感染羊种布鲁氏菌已成为一个重要问题。与一些传统观念相反，羊种菌感染牛之后对人仍有很强的致病性。牛被感染是特别严重的问题，因其可产出大量被污染的牛奶，甚至流产或感染的牛犊都可以造成广泛的环境污染。

牛种菌是导致感染最普遍的布鲁氏菌，但很少导致人患病。人感染牛种布鲁氏菌常表现为亚临床状态，一旦发病，症状也比羊种菌和猪种菌感染都要轻。牛是迄今最常见的牛种菌宿主，但在一些地区野牛、水牛、骆驼、犬和牦牛也是重要的宿主。

与羊种菌和牛种菌相比，猪种菌致病更受限制。它作为重要的传染源，导致的疾病严重程度与羊种菌相同。由于猪种菌的生物变型（实验室检测亚型）较多，造成了其来源和毒力的多样性。

猪种菌 1 型、2 型和 3 型都与猪有关，其中 2 型猪种菌还与野兔有关。这种生物变种对人有低致病性，但 1 型和 3 型有较强毒力并可导致严重疾病。4 型猪种菌与阿拉斯加、加拿大、俄罗斯北部的驯鹿和麋鹿有关。很少有报道 4 型猪种菌引起人患病。没有人自然感染 5 型猪种菌的报道。

许多国家犬种菌导致犬的普遍感染，但很少感染人。报道的病例通常发病缓和。

布鲁氏菌可感染多种野生动物，但很少通过野生动物传播给人。

3.1.2 人间布鲁氏菌病的传播

布鲁氏菌病可能的传播途径包括：人传人、从污染的环境感染、由于直接接触被感染的动物引起的职业暴露、食源性传播。

3.1.2.1 *人传人* 人传人极其罕见。偶有报道，间接证据显示亲密身体接触或性接触可作为传播途径。

人传人更多的潜在意义是通过献血或器官移植造成的传播。特别是骨髓移植有重大风险。建议对血液和器官捐赠者进行布鲁氏菌病筛查，以排除近期感染史。患者将布鲁氏菌病传播给陪护几乎不可能，但也要采取预防措施。实验室工作人员处理患者样本时有巨大风险。

3.1.2.2 *从污染的环境感染* 除非频繁发生布鲁氏菌病，否则很难证明感染来自污染的环境。病畜（尤其是流产后），经过人口稠密区或紧邻房屋时能严重污染街道、庭院和集贸市场。人暴露于污染的尘土、干粪等可能导致吸入性布鲁氏菌病。皮肤或结膜感染也可导致接触性布鲁氏菌病。井水等水源也可能被近期流产动物或污染地区流经的雨水污染。

布鲁氏菌可在尘土、粪便、水、泥浆、流产胎、土壤、肉及奶制品中长期存活，存活的时间取决于许多因素，例如介质的性质、布鲁氏菌的数量、温度、pH、阳光、其他微生物数量。表 3 中举出了一些例子。

表 3 牛种和羊种布鲁氏菌在不同介质中的生存期

介质	温度或环境	生存时间
牛种		
固体表面	＜ 31℃，阳光	4 ～ 5h
自来水	～ 4℃	114 天
湖水	37℃，pH 7.5	＜ 1 天
湖水	8℃，pH 6.5	＞ 57 天
干土壤	～ 20℃	＜ 4 天
湿土壤	＜ 10℃	66 天
粪肥	夏季	1 天
粪肥	冬季	53 天
农场动物粪液	粪池环境温度	7 周
农场动物粪液	粪池 12℃	＞ 8 个月
羊种		
肉汤	pH ＞ 5.5	＞ 4 周
肉汤	pH 5	＜ 3 周
肉汤	pH 4	1 天
肉汤	pH ＜ 4	＜ 1 天
软奶酪	37℃	48 ～ 72h
酸奶	37℃	48 ～ 72h
奶	37℃	7 ～ 24h

3.1.2.3 *职业暴露* 某些职业是布鲁氏菌病感染的高危职业。包括从事与牛、绵羊、山羊、猪等家畜有关工作的人，如农民、农场工人、动物饲养员、牧民、剪羊毛者、兽医和配种员，由于与病畜直接接触或暴露在严重污染环境下而存在危险。感染可能通过以下途径发生：吸

入性、结膜感染、误饮、特别是通过切口或擦伤的皮肤感染、活疫苗的意外接种。

家畜圈养在人的住房附近时，家庭暴露与职业暴露可能是分不开的，因此，农民和饲养员的家人也会存在风险。在某些地区，家畜被饲养在庭院中，尤其在恶劣的天气时，甚至被带到住房内。若近期有动物流产，这会导致全家人的感染。用干粪作为燃料也可以感染全部家庭成员。应当注意到，在畜间布鲁氏菌病疫情暴发之后，家庭或村落中常出现布鲁氏菌病聚集性病例，这一般与食用共同的污染食物有关。

儿童因为将幼畜或病畜作为宠物，故而存在特别的感染风险。有些地区成年人可能具有免疫力或者存在慢性感染，因此，儿童可能是唯一出现急性症状的人群。

畜产品加工者患布鲁氏菌病的风险很高，包括屠夫、肉贩、肉类加工者、胎牛血清收集者、皮毛加工者、乳制品工人。环境的污染可通过呼吸、食入、皮肤黏膜接触等造成危害。

在维护农场的房屋、畜产品加工车间和工厂时，所雇用的员工作为职业暴露人群常被忽视，但他们因处于污染的环境而可能存在相当大的风险。

培养布鲁氏菌的实验室人员存在特殊风险。在某些布鲁氏菌病疫情得到控制的国家，可能忽视这个潜在的危险。然而，在输入性病例的诊断过程中，直到布鲁氏菌被培养出来，才让人高度怀疑发生了实验室感染。快速识别集成检测系统曾将布鲁氏菌误判为莫拉菌属，造成过严重后果。细菌培养过程中，特别是离心时出现容器破损，则吸入气溶胶风险最大。

制备和使用 S19 和 Rev 1 这类活疫苗时也存在风险。粗糙型的 RB51 菌苗似乎是低致病性的，但仍存在意外接种的潜在风险，并对利福平耐药。应尽可能避免使用强毒株作为诊断抗原。

3.1.2.4 *食物传播* 食物传播通常是城市人群感染布鲁氏菌病的主要原因。食用新鲜牛奶或未经热处理的奶制品是大部分人群的主要感染来源。被羊种布鲁氏菌污染的牛奶、绵羊奶、山羊奶、骆驼奶尤其危险，因为这些奶每天被大量饮用，并可能含有大量布鲁氏菌。用这些奶制作的黄油或冰淇淋也存在高风险。在地中海和中东国家，添加了凝乳酶后的绵羊奶或山羊奶制作的软奶酪是常见的传染源。奶酪的制作过程实际上浓缩了布鲁氏菌，这些细菌能在其中存活数月以上。这些奶酪应在阴凉的环境下存储至少 6 个月再食用。由奶和丙酸发酵而制作的硬奶酪存在风险较小。同样，酸奶和酸奶酪的风险较小。布鲁氏菌在 pH 低于 4 的环境下快速死亡，当 pH 低于 3.5 时则极快死亡。应遵守良好的卫生规范，否则，运输或加工污染奶或其他原料的设备，也能污染其他产品。

肉制品较少与感染有关，主要因为肉制品很少被生吃。这在屠夫和屠宰场工人中已是惯例。肝、肾、脾、乳房和睾丸中的布鲁氏菌浓度比肌肉组织都高，在某些国家，用这些器官制作的生的或未熟的菜肴可能被食用。饮用新鲜的血液或血奶混合物，均有潜在危险。

在许多国家，“健康食品”的消费变成一种时尚，这些食品常常包括未经高温消毒的牛奶及奶制品，可能因此造成特殊的风险。很难让人们认识到此类“健康”产品可存在危险。

生的蔬菜可被感染动物污染而出现风险。在一些流行区，游客食用“民族”食品的风险更高。

由于疾病或服用抗酸药、H_2 拮抗药而导致胃酸缺乏的人，通过食用被污染食物而患布鲁氏菌病的风险加大。

因疾病或使用免疫抑制药而处于免疫缺陷状态的个体，其患严重布鲁氏菌病的风险也可增加，尽管这很难量化。

3.1.3 季节因素

处于温带或寒带的国家，布鲁氏菌发病率存在显著的季节性变化，大多数病例出现在春季或夏季，这与家畜流产或分娩的波峰周期一致。此时也是照料家畜和食用动物乳汁的最佳时期，因此处于暴露的最高水平。与牛相比，季节影响对绵羊或山羊布鲁氏菌病更为明显，这可能是由于牛的泌乳期较长的原因。

在动物繁殖贯穿全年的热带亚热带地区，布鲁氏菌病发病率不受季节影响。

3.1.4 年龄和性别分布

在工业化国家和注重预防食源性布鲁氏菌病的国家，布鲁氏菌病是非常主要的职业病并且多数病例是 20 ～ 45 岁的男性，通常是牛种或猪种布鲁氏菌造成感染。羊种布鲁氏菌流行的国家和地区，特别在绵羊和山羊奶制品贩卖和流通的情况下，较难执行卫生措施，此时所有人都存在被感染风险，妇女和儿童发病较多。在游牧民族中，尽管很多人会因为长期感染而存在后遗症，但成年人往往在年龄较小时已暴露感染，因此，不表现出急性期症状，这种情况下，急性病例中儿童占很高比例，布鲁氏菌病是一个主要的儿科问题。

3.1.5 旅行感染布鲁氏菌病

游客或商人到流行地区可能感染布鲁氏菌病，通常由于食用未经高温消毒的奶或奶制品。旅行者也能向本国输入污染的奶酪和其他奶制品并感染其家庭、朋友等。当前在北美和北欧地区输入性病例占急性期布鲁氏菌病的绝大多数。

3.1.6 生物恐怖主义

由于布鲁氏菌气溶胶相对稳定，并且低剂量就能造成感染，因此，在某些国家资助下，羊种和猪种布鲁氏菌已被实验性地研发成为生物武器。它可用来攻击人或动物，尤其在疾病非流行区影响巨大。病原体可以源自世界上许多地区的自然环境。卫生和畜牧部门应了解这种潜在的传染源。

人布鲁氏菌病流行病学要点

- 牛、绵羊、山羊和猪是布鲁氏菌主要的宿主。
- 通过职业或环境接触感染动物及其制品而传播给人。
- 食物传播是主要感染来源，用生奶和未经消毒的奶制作的奶酪存在高风险。
- 布鲁氏菌病是旅行相关性疾病。
- 血液或移植的器官／组织是可能的传染源。
- 人际传播极其罕见。

3.2 动物布鲁氏菌病流行病学

动物布鲁氏菌病的影响会随着宿主种类的不同而变化。牛通常感染牛种菌，然而羊种菌和少数的猪种菌也能感染牛，并且传播方式与牛种菌类似。多数羊种菌和猪种菌毒力强、动物排菌数量多，因此对于人特别危险。

牛和其他牛科动物间传播布鲁氏菌常是通过接触流产物。牧场和畜舍可被污染并且病原体除吸入、结膜感染、皮肤污染外最可能被食入，接触污染的挤奶杯也能造成乳房感染。采集初乳饲喂牛犊也可造成传播。性传播在牛布鲁氏菌病流行病学中影响较小，然而，人工授精能传播疾病，因此，精液必须来自健康动物。

羊种菌通常会感染绵羊和山羊。绵羊附睾种菌也可感染绵羊，但这对于人患病几乎没有意义。绵羊和山羊间的布鲁氏菌传播方式与牛相似，但性传播影响更大。牲畜混群和购入未检疫的牲畜促使疾病进一步传播。共用种公畜也能促使疾病在农场间传播。在市场或集市中动物间的接触以及夏季牧场的迁徙是重要的传播促进因素。在寒冷气候时，习惯在封闭的房屋中饲养动物，这也促进了传播。

直接接触流产母猪、食用污染食物或暴露污染环境导致猪布鲁氏菌病的传播。然而，性传播尤为重要，通过共用种公猪或购买感染牲畜可造成农场的输入性传播。

对于所有物种，只要遵循推荐的程序，胚胎移植是安全的。

犬布鲁氏菌病可能是犬类饲养场的主要问题。接触近期流产动物和被流产物（或排泄物）污染的环境（或食物）都能造成传播。性传播也是重要的播散途径，雄性可通过精液排泄大量病原菌。尿液也可含有病原菌且对人造成潜在威胁。犬群中出现犬种布鲁氏菌的国家，似乎人极少感染犬种布鲁氏菌。

要知道，犬通常因食用反刍动物或猪的流产胎、胎盘等而感染牛种、羊种和猪种布鲁氏菌。他们能够排出病原菌并给人或家畜带来严重危害。

驯鹿的布鲁氏菌病由猪 4 型布鲁氏菌造成。流行病学与牛布鲁氏菌病相似。通常的途

径能传播给人，然而人食用生的或未煮熟的驯鹿骨髓也可感染。

牛、绵羊、山羊和猪中，成畜对布鲁氏菌病最易感。幼畜常有抵抗力，应该注意存在隐性感染且这些动物在成年后可造成危害。

易感性也会受到动物种类的影响，尤其是绵羊。产奶动物似乎对羊种布鲁氏菌最易感。尽管已证明基因决定个体动物的易感性，但尚无不同品种的牛易感性有差异的明确记载。天然抵抗力的多态性与单核细胞蛋白(NRAMP)基因有关，显著影响牛和猪的布鲁氏菌病易感性。然而，牲畜管理对降低感染风险更为重要。由于宫内感染或产后早期感染，可致农场动物中出现隐性感染。这些动物能够终生带菌，直到第一次分娩或流产，血清学检查才会出现阳性。约 5% 感染的奶牛妊娠后会呈现隐性感染。其他品种的严重程度未知，但曾有绵羊感染具有潜伏性的报道。

后天免疫对易感性有实质影响。用 S19 或 RB51 免疫牛，或用 Rev 1 免疫绵羊或山羊能够降低家畜对相应细菌的易感性 1000 倍或更多。S19 不能保护牛被羊种布鲁氏菌感染，然而使用 Rev1 免疫牛的资料又很少。在某些羊种布鲁氏菌流行地区 Rev 1 疫苗的效力也被质疑。疫苗来源必须可靠且有国际认可。可能存在用羊种菌免疫不产生抗体的情况。不同疫苗株的保护率可能会有变化，目前，对于羊种菌 Rev 1 是最有效的疫苗，并且在许多国家取得了很好的效果，建议在小型反刍动物中出现难以控制的羊种菌感染时使用。

动物布鲁氏菌病流行病学要点

- 牛通常感染牛种菌，然而羊种菌和少数的猪种菌也能感染牛。
- 羊种菌主要感染绵羊和山羊，猪种菌感染猪。
- 随着直接接触和流产后污染环境而出现传播。
- 性传播和（或）人工授精传播也很重要。
- 可出现血清学反应阴性的潜伏感染。

4. 诊断

布鲁氏菌的分离鉴定可为布鲁氏菌病诊断提供明确依据，有益于流行病学研究和监控动物免疫规划进程。接触布鲁氏菌的实验室人员存在风险。必须遵守当地处理布鲁氏菌的法律规定和实验室最低安全标准。详见“6. 人布鲁氏菌病的预防”。诊断试验的国际标准信息，请参考《陆生动物的诊断试验与免疫手册》，世界动物卫生组织，2004。

4.1 人布鲁氏菌病的诊断

由于布鲁氏菌病临床表现多样，因此诊断不能单独依靠临床证据，必须进行血清学和

病原学检验。接诊生活在疫区或近期曾到布鲁氏菌病流行国家旅游（旅游相关性疾病）的发热患者时，医生必须意识到患者被布鲁氏菌感染的可能性。因此，正确采集病史对鉴别诊断至关重要，并且要着重询问一些基本的问题（职业、食物摄入、接触动物和疫区旅行史）。此外，必须进行快速筛查试验。虎红平板试验可用于快速筛查，但结果应经病原学和其他血清学试验确认。如果存在病史和临床症状，而筛查试验阴性，则建议使用其他的检测方法。认真遵守上述事项有助于避免误诊。

4.1.1 病原学诊断

从患者体内分离到布鲁氏菌是确定布鲁氏菌感染的唯一明确证据。尽管从骨髓、脑脊液、伤口、脓液等可以分离出布鲁氏菌，但细菌培养用得最多的还是血液。有报道，培养前浓缩和溶解部分白细胞有助于提高出菌率。

推荐使用同一容器中既有固体又有液体的双相培养方法进行血液培养，即卡斯塔涅达法。这不需要传代，因此，降低了实验室感染风险。血清葡萄糖肉汤加相应的固相培养基常被推荐，然而布鲁氏菌在多数用于血培养的高质量蛋白胨培养基上也可以生长。无菌条件下采集的人血液样本不是必须使用选择性培养基培养。恒温培养应向空气中添加 5% 的 CO_2。改良的半自动培养方法（BACTEC 9204 和 Bac/Alert）大大缩短了检测时间；这些方法在培养第 3 天就能检测到布鲁氏菌，然而该方法与传统方法比较的报道有限。需要注意的是，通过传统血液培养方法检测阳性的样本，运用初期的血液培养方法（BACTEC NR 730）反而检测呈阴性。

常规的双相血液培养法在恒温培养前 4 天很少呈阳性，多数血液培养在第 7 ～ 21 天出现阳性，有 2% 的阳性出现在第 27 天之后。因此，恒温培养至少持续 45 天后，才可认定为阴性。定期将培养瓶中的肉汤血液混合物覆盖到固相上，以减少传代。如果 1 周后仍未生长，需重复以上操作。建议用改良的抗酸染色法涂片、染色，这个过程能够将布鲁氏菌与可能的革兰阳性球菌、皮肤污染菌以及血培养基中的杂质相区分。

在地中海国家，很少从病例中分离到牛种布鲁氏菌，此原因未知。例如，在西班牙从人体分离的 2107 株布鲁氏菌超过 98% 都是羊种菌。发热病例血清培养阳性率可高达 86.5%。在低热或不发热的病例中阳性率则下降至 75% 和 28.5%。复发病例同样如此。然而两项大范围研究显示，31.8% 和 41.9% 的羊种布鲁氏菌是从无发热症状病例中分离出来的。

“属”水平的布鲁氏菌可根据菌落形态、革兰或印记涂片染色状态、氧化酶和布鲁氏菌特异抗血清玻片凝集试验结果进行分离筛查，分子识别方法也可以进行分离筛查，例如采用特异插入序列引物和基因片段的聚合酶链反应方法，引物包括 IS 711 或 IS 650，基因片段包括 *16S-23SrRNA*，*BCPS31* 和 *omp 2a* 基因。分离到的布鲁氏菌应提交给参比实验室做精确的种型鉴定，因为这可以提供非常有价值的流行病学信息。

近期有学者提议用聚合酶链反应方法直接在血液中检测布鲁氏菌。该方法是否能够取代传统血液培养方法还需更多试验证明。将这类试验作为常规实验室检测之前，必须确认其

敏感度、特异度和可重复性。进行这类试验操作前须制定有效的控制规程，防止来自实验室环境的细菌 DNA 或放大复制子的样本污染。

4.1.2 血清学诊断

4.1.2.1 *抗原和免疫球蛋白在诊断中的意义* 用于诊断人布鲁氏菌病的主要布鲁氏菌抗原是外层细胞膜和内部（细胞质）蛋白的光滑脂多糖（S-LPS），它是免疫显性抗原，还是一种分子，该分子能携带与其他革兰阴性菌起交叉反应的表位，这些革兰阴性菌包括小肠结肠炎耶尔森菌 O：9，大肠埃希菌 O：157，土拉弗朗西斯菌、沙门菌 O：30 及霍乱弧菌等。

试管凝集试验（SAT）或根据其改良的微滴定平板试验，用高温或苯酚（石碳酸）灭活的光滑菌体来检测 S-LPS 抗体。当试验使用含有 S-LPS 的提取物时，其他检验方法也能检测到抗原抗体反应，例如酶联免疫吸附试验。由于这是免疫显性抗原，因此，用 S-LPS 无胞质蛋白制剂来检测抗体。使用胞质蛋白的重要一点是从未发现与上述细菌存在显著的血清学交叉反应（然而能与布鲁氏菌基因相近的细菌出现交叉反应，如苍白杆菌等），因此，这些蛋白可在 S-LPS 水平上用以区分布鲁氏菌感染还是其他交叉反应细菌感染。

人对布鲁氏菌免疫应答的特点是感染初期产生 IgM 类抗体，随后长期分泌 IgG 类抗体。用 S-LPS 的酶联免疫吸附试验可以测量随着感染和治疗后免疫球蛋白的演变。人们发现酶联免疫吸附试验检测 IgM 与血清凝集滴度之间有很好的相关性。在一项研究中，治疗后 IgM 抗体比 IgG 抗体滴度下降更快。25% ～ 50% 的急性期布鲁氏菌病患者治疗后 1 年才出现 IgM，这些患者 85% 在临床痊愈后 18 个月 IgG 抗体滴度仍较高，但复发病例中，IgG 抗体滴度增高而 IgM 不增高。IgA 滴度与 IgG 滴度基本持平。与此相反，使用试管凝集试验和二巯基乙醇（2-ME）或二硫基苏糖醇（DDT）凝集试验的另一研究显示，成功治疗的布鲁氏菌病病例 2-ME 凝集试验（不破坏 IgG 和不破坏 IgM）中 IgG 抗体滴度快速下降，然而，用试管凝集试验测量血清，低水平的 2-ME（破坏 IgM 或 IgA）凝集仍长期存在。应该注意的是改良检验对 IgM 不完全特异，因此必须慎重解释其结果。酶联免疫吸附试验优先检测特异性免疫球蛋白，这是 2-ME 和酶联免疫吸附试验检测 IgG 结果明显不同的部分原因，此外，酶联免疫吸附试验中少量 IgG 类抗体不凝集现象也是造成这两种实验结果明显不同的原因。布鲁氏菌抗体滴度与临床感染过程相关，这点十分重要。所以必须认识到单个检验方法只能测量诸多抗体中的一类。

理论上，急性期布鲁氏菌病患者首先产生的免疫球蛋白是 IgM，随后在未接受治疗的患者体内产生 IgG。缓慢发病的病例只有在患病晚期或复发时才可见 IgM 应答反应。患病过程中或复发病例的 IgM 应答较晚被发现。凝集滴度（IgM，IgG，IgA）应在成功治疗后下降；否则，有必要评估病人复发或慢性局部感染的可能性。复发时 IgG 和 IgA 滴度上升。

4.1.2.2 *光滑性脂多糖抗体的血清学检测* 虎红平板凝集试验是当前推荐的快速筛查试验，但其试验结果应由其他试验来确证，特别在动物布鲁氏菌病高发地区。虎红平板凝集试

验的灵敏度超过 99%，但在下述情况下可能出现假阳性反应：血清来自感染小肠结肠炎耶尔森菌 O∶9 或其他交叉反应抗原的患者或曾与光滑型布鲁氏菌有接触但未发病的健康人。

使用标化抗原的试管凝集试验对诊断人布鲁氏菌病非常有意义，可以用国际单位表达滴度，且与临床感染阶段相关性较好。为使该检测信息量更多，应用最终浓度为 0.05M 的 2-ME 磷酸盐缓冲液或最终浓度为 0.005M 的二硫基苏糖醇进行平行凝集试验，因为它们能够破坏 IgM 和 IgA 的凝集活性。

试管凝集试验什么滴度能表明存在活动性感染？该问题尚未得到解决。不可能预测每个病例产生的个体反应，更不用解释为什么一些患者凝集滴度高，而其他人患病期间凝集滴度较低。例如，对 238 例布鲁氏菌病患者研究发现，如果将 1∶80（100U）作为诊断滴度，29.2% 的病例被认定为阴性，相同的研究中发现，3.4% 的患者血清滴度为 1∶10（12U）甚至更低。作为一个指南，如果病人出现症状和体征，滴度为 1/160（200U）或以上更有明确诊断价值。一些学者考虑到在动物布鲁氏菌病高发地区，诊断滴度应该比这更高，因为许多无症状个体将会有该水平的血清滴度。这种情况下，试管凝集试验的价值严重受限。即便血清滴度变化高度提示感染，但这在现实中很少见，因为极少在感染足够早期采集血清样本。

有报道，IgG 酶联免疫吸附试验的结果与抗人球蛋白试验结果高度相关。抗人球蛋白试验（和 IgG 酶联免疫吸附试验）比其他凝集试验保持阳性的时间更长。若从感染到诊断间隔时间很长，抗人球蛋白试验滴度通常就很高。可总结如下：急性期布鲁氏菌病，抗人球蛋白试验通常比试管凝集试验滴度高 4 ～ 16 倍，然而随着未治疗患者病程的进展，滴度可高达 16 ～ 256 倍。

虽然免疫荧光检测和放射免疫荧光检测曾被使用，但研究免疫球蛋白类型分布的最适合方法是间接酶联免疫吸附试验。资料分析显示，酶联免疫吸附试验用于测量 IgG 抗体并且能用 S-LPS 和抗 IgG 轭合物的间接酶联免疫吸附试验取代抗人球蛋白试验。尽管用 S-LPS 酶联免疫吸附试验是很有前景的检验方法，但也存在些问题，包括试验的标准化问题、商业试剂质量和试验结果说明的参差不齐、特别是单独以光密度读数为依据时，引发的实验室间可比性等问题，都需要靠建立标准参照物来解决。

鉴于技术复杂（难度高于试管凝集试验和酶联免疫吸附试验）和标准化问题，不推荐在小型实验室日常应用补体结合试验，尽管该试验很实用。经验显示：① 91.7% 的患者补体结合试验和试管凝集试验阳性；②患病第 4 或第 5 个月，补体结合试验比试管凝集试验滴度高；③约 4.6% 的患者在患病的最初几天或几周补体结合试验结果阴性而试管凝集试验滴度明显；④约 3.7% 的慢性期或已康复患者试管凝集试验结果阴性而补体结合试验滴度明显。

4.1.2.3 *胞质蛋白抗体的血清学检测* 可通过免疫凝胶电泳（CIEP）、酶联免疫吸附试验和 Northern 印迹法研究抗布鲁氏菌胞质蛋白的抗体。人们通过免疫凝胶电泳发现，抗 2-ME 蛋白抗体的血清与抗人球蛋白试验（Coomb's）高滴度血清一样，能产生大量的蛋白沉淀线

并且蛋白抗体滴度更高。显然，这些患者的病程较长，这意味着沉淀线数随着患者未接受诊断和治疗的时间增加而增加。酶联免疫吸附试验研究显示，抗 S-LPS 抗体可能出现在与光滑型布鲁氏菌曾有接触但未发病的人体内，该选择蛋白抗体表明存在活动性感染。

在一些持续感染或复发病例中抗蛋白抗体滴度仍然很高，而在康复患者中抗蛋白抗体消失，Northern 印迹法也取得相似的定性结果。这些方法的缺点包括非量化、解释过于主观、缺少验证数据和参照试剂。

4.1.3 布鲁氏菌脑膜炎和脑膜脑炎的诊断

理想情况下此类患者应从脑脊液中培养布鲁氏菌，考虑到多数情况下常规培养都呈阴性结果，所以必须对脑脊液进行血清学检测。未影响中枢神经系统患者的脑脊液中不产生抗体。与此相反，那些神经型布鲁氏菌病患者中，脑脊液包含低滴度的 S-LPS 和胞质蛋白抗体，这些抗体很容易通过虎红平板凝集试验和对流免疫电泳试验检测到。脑脊液通常是无菌的并且分析结果显示脑脊液中 IgG 和淋巴细胞增多。

4.1.4 皮内试验

皮内注射布鲁氏菌特异抗原的迟发型超敏反应阳性表示过去曾经感染，但并不能说明目前的状况。尽管一些国家曾使用过，但皮内试验不建议用于诊断。使用未确认或未标化抗原可能干扰后续血清学检测。

4.1.5 结论

人布鲁氏菌病血清学诊断可以用 S 期全细胞检测。推荐使用如下试验：RBT，单纯或使用 2-ME 或 DTT 缓冲的 SAT，Coomb's 凝集试验，CFT 和 ELISA。例如联合使用 SAT 与 Coomb's 能评估诊断时疾病所处的阶段。合适的 IgM 或 IgG 与 S-LPS 特异性结合的 ELISA 能够取代正式建立的试验，但需要进一步标化和确认。其他方法可能有效，但特异性较差且缺乏充分地评估。

人布鲁氏菌病诊断要点

- 对于急性布鲁氏菌病，从血液或其他组织中分离布鲁氏菌至关重要。
- 培养通常阴性，特别是病程较长的情况下。
- 血清学是最常用的诊断方法。
- 推荐使用虎红平板凝集试验、试管凝集试验和酶联免疫吸附试验。
- 不同的 IgM 和 IgG 的检测方法能区分活动性感染和既往感染。
- 血清学反应假阳性偶有发生。
- 皮内变态反应能够揭示曾经暴露，不能证明活动性感染。

4.2 动物布鲁氏菌病诊断

动物布鲁氏菌病的诊断和控制必须基于群体水平。一些感染动物可能潜伏期很长，个体可在感染后很长时期内血清学阴性。一个或多个感染动物的确诊是群体中存在感染的充分证据，此时其他血清学阴性的动物可能处于潜伏期且存在风险。

诊断试验共分两大类：病原检测和抗原的免疫应答检测。分离到布鲁氏菌是动物感染的明确证据，但不是在所有感染动物体内都能分离到布鲁氏菌，而且并非所有方法和设备都有效。抗体的检测和超敏反应只能提供初步诊断，但现实中这些检测是最可行和最经济的手段。由于接种疫苗等因素，血清学检测可以出现假阳性反应，这在解释结果时必须牢记。同样，皮肤超敏反应仅表明以往曾暴露于布鲁氏菌，不一定存在活动性感染，也可能因接种疫苗导致。

在多数防控策略中，疫苗极其重要和有效，但其缺点是使用后会混淆皮内试验和血清学检测的诊断结果。少量免疫动物的抗体滴度可长期持续，且这类动物数量会随着免疫而逐年增加。为了减少此类问题，通常对＜6 个月的牛犊进行免疫。如果减少剂量也可以在成牛中使用，特别是在结膜接种的情况下。当前没有能区分动物免疫和感染的普及检测方法，尽管一些试验处于评估阶段。

免疫时严格控制畜龄、疫苗质量和免疫动物等至关重要，否则无法进行正确的血清学诊断。当发病率降到很低水平或免疫的成本效益和成本效果分析弊大于利时，可暂停免疫。

4.2.1 细菌学方法

布鲁氏菌的分离鉴定可为布鲁氏菌病诊断提供明确依据，有益于流行病学研究和监控动物免疫规划进程。应该注意所有污染物都存在严重风险，采集、运输、加工、处理过程中必须采取充分的防范措施。

4.2.1.1 *涂片染色* 胎盘小叶、阴道分泌物、胎胃内容物的染色可以用改良抗酸染色法或 Kosters 法。细胞内存在大颗粒物，弱抗酸的布鲁氏菌形态是推定布鲁氏菌病的证据。必须考虑其他病原在显微镜下与布鲁氏菌相像，例如伯纳特式立克次体或衣原体（彩图 3）。

4.2.1.2 *培养* 流产或分娩期间布鲁氏菌最容易被分离，但也可尝试在解剖时进行分离。

分娩时大量布鲁氏菌被排出，可使用选择性培养基从阴道黏液、胎盘、胎儿胃内容物和奶等材料中进行培养。避免材料被粪便和环境污染至关重要，这样更能成功分离到布鲁氏菌。如果其他材料难以获得或严重污染，胎儿胃内容物反而是布鲁氏菌的极好来源。

某些情况下，可通过解剖分离布鲁氏菌。合适的材料包括乳腺上淋巴结、髂内淋巴结、咽后淋巴结、乳房、睾丸和妊娠子宫。

奶样本在低速离心前应置 4℃过夜。将奶油和沉淀涂抹到至少 3 个固体选择性培养基平板表面。胎盘样本应在现场选择污染最少的部分切下一片子叶。在实验室，要用乙醇火焰燃

烧过的剪刀或手术刀切割子叶，然后将切割面涂抹在 3 块选择性培养基上。其他固体组织可以用类似的方式处理，更理想的方法是，培养前浸软组织物，在添加少量无菌水后通过手动或搅拌机均质研磨。收集胎儿胃内容物时，打开腹腔后，用热压舌板烤焦胃表面并且用巴斯德吸管或注射器吸引液体内容物。

可通过培养特性、形态、革兰染色和阳性血清凝集（彩图 4 和彩图 5）暂时来鉴定布鲁氏菌菌落。如果条件允许，也可使用基于 PCR 的分子学鉴定方法。最终鉴定疑似菌落只能使用布鲁氏菌参比中心的有效技术。

4.2.2 血清学方法

血清或奶中特异抗体的检测仍然是诊断布鲁氏菌病最实用的方法。最有效和最具成本效益的方法是先用廉价、快速、敏感性高的检测方法筛查所有样本，然后用更复杂、特异和确切的方法对筛查出的阳性样本进行最终诊断（彩图 5 和彩图 6）。

有必要使用标化抗原，即抗牛种布鲁氏菌的第二国际血清标化的抗原。每一批测试应包括血清质量控制，如果不符合质量控制标准应重复检测。

血清学结果必须以布鲁氏菌病发病率、免疫和其他感染导致的假阳性反应为背景进行解释。所有实验室诊断，必须对个体动物身份、样品号进行“追踪调查”，进而最终确定动物与检测结果之间的联系。

4.2.2.1 *虎红平板试验（RBT）* 虎红平板试验是诸多缓冲布鲁氏菌抗原检测方法之一，其原理是在低 pH 条件下，IgM 抗体与抗原结合能力明显降低。虎红平板试验、平板凝集试验和卡片试验等在全世界布鲁氏菌病血清学诊断方面发挥了重要作用（彩图 7）。

虎红平板试验是简单斑点凝集试验，在平板上将染色抗原和血清各一滴混合，产生凝集即为阳性。该检测用于筛查很好，但由于敏感性较高，不适用于诊断动物个体，尤其是诊断被免疫动物。操作过程可以实现自动化，但需定制设备。

4.2.2.2 *酶联免疫吸附试验（ELISA）* 酶联免疫吸附试验灵敏度和特异度很好，稳定性强，操作简单，设备需求少并且有市售试剂盒。与补体结合试验相比更适合小型实验室，该技术当前用于诊断多种动物和人布鲁氏菌病。尽管在原则上，酶联免疫吸附试验可检测所有动物和人的血清，但由于试验方法不同，实验室之间的结果也会存在差异，不能够完全标准化。筛查时检测通常用一个稀释度，应注意的是，尽管酶联免疫吸附试验比虎红平板试验更敏感，但有时检测不出虎红平板试验检测阳性的样本。酶联免疫吸附试验比虎红平板试验或补体结合试验在特异性上稍高一点。

4.2.2.3 *试管凝集试验（SAT）* 试管凝集试验广泛应用于布鲁氏菌病的诊断，尽管操作简单、成本低廉，但缺乏灵敏度和特异度，意味着只在无替代方法的时候才使用。

4.2.2.4 *补体结合试验（CFT）* 补体结合试验具有良好的敏感性和特异性，但由于其

方法复杂，操作需要良好的实验室设施和训练有素的人员。如果具备这些条件，在保证质量的前提下定期实施该检测，结果会较为理想。

一些感染动物血清中存在大量非补体固定抗体竞争结合抗原，故进行低倍稀释时不能固定补体而出现前带现象，对其进行高倍稀释后补体则被固定。如果仅以单一的稀释法筛选，上述样本将被漏掉，因此，必须滴定每一份血清样品。

即使在缺乏抗原等情况下，血清样本被细菌污染或其他因素固定或破坏补体也会引起试验阳性反应，此种“抗补体”反应使检测无效或不能获得检测结果。

4.2.3 补充试验

其他血清学检测方法也被使用，如依沙吖啶试验或 2-ME 试验都是改良的试管凝集试验，虽然更特异，但存在许多缺点。目前，标准实验室都不建议使用这些方法。

4.2.3.1 *乳汁检测* 在产奶动物中，乳汁因易获得又廉价而成为理想的检测物，可以定期重复检测并能较好反映血清抗体。来自搅拌器或收集罐的乳汁可用于筛查畜群中是否存在感染动物，然后用血液检测来确诊。该方法非常有效并且在检测产奶动物中经常使用。

4.2.3.2 *乳环试验*（MRT） 乳环试验是简单有效的方法，但只能用于牛奶。将一滴苏木精染色抗原与少量牛奶在玻璃或塑料管中混合，如果牛奶中存在特异性抗体，会与抗原结合，并与奶油形成蓝色的环，上升至牛奶柱的顶部。该试验相当敏感，但也可能检测不出大群牲畜中的个别感染动物。该试验非特异性反应常见，尤其在无布鲁氏菌病地区，乳汁酶联免疫吸附试验比乳环试验更加特异。

4.2.3.3 *乳汁酶联免疫吸附试验* 酶联免疫吸附试验可用于检验散装奶，具有极好的敏感性和特异性，多数情况下，能完成大群牲畜中个别感染动物的检测。

4.2.3.4 *荧光偏振法* 该技术需要特殊试剂和读取设备，其敏感性和特异性超过其他方法。然而对该方法评价有限，并未广泛应用，其整体价值被评估之前，需要进一步补充信息。

4.2.3.5 *皮内试验* 皮内试验使用布鲁氏菌素 INRA 和布鲁氏菌基因 OCB 等标准抗原，可用于无布鲁氏菌病地区的畜群监测。该试验既敏感又特异，但免疫动物中能出现假阳性反应。

4.3 除牛外的布鲁氏菌病诊断

上述过程主要用于牛布鲁氏菌病诊断，然而细菌学诊断适用于所有物种。对于个别物种血清学试验方法改良如下。

4.3.1 绵羊和山羊

虎红平板试验通常用于筛查羊种布鲁氏菌产生的血清抗体。建议将抗原凝集滴度调整

到质控血清的最高敏感度。该检验对牛不太敏感，可能不会检测到一些被感染的动物。最好与确证试验结合使用。

试管凝集试验已广泛使用 5% 氯化钠溶液作为稀释剂，但敏感性和特异性较低。

微量凝集反应的敏感性和特异性较高，只有在其他更精密的试验不能开展时被推荐使用。其抗原标化时，被标化抗原与滴度为 1：650 的牛种布鲁氏菌第二国际标准血清反应会产生 50% 凝集。

应注意凝集法对非特异性凝集素和交叉反应抗体特别敏感。凝集法在感染急性期更为敏感并且受免疫影响很大，应该仅在没有替代方法时使用。补体结合试验优于凝集法，但其敏感性和特异性有限，并且被认为是补充试验，而不是确证试验。如果半自动方法可行，可用其进行筛查，血清应 62℃灭活 30min。在补体结合试验中显示，免疫产生的血清抗体滴度下降比自然感染时快得多。有报道酶联免疫吸附试验的敏感性和特异性优于其他试验，但关于此类验证有限。绵羊和山羊乳汁不适用乳环试验，而适用酶联免疫吸附试验。补体结合试验也可用于乳清样本，但技术上要求苛刻，不再推荐使用。也可理解为目前在动物个体水平上，没有检测绵羊和山羊布鲁氏菌病有效、可靠的血清学方法，故实施诊断和控制应建立在群体水平上。

在羊群或牛群中，皮内试验用于检测布鲁氏菌抗原的迟发性过敏反应，但也受免疫的影响。纯化抗原由布鲁氏菌蛋白混合物制备，其中不应包含光滑脂多糖（S-LPS），以避免影响血清学试验。此方法用于监测无布鲁氏菌病畜群状态，特别是未经免疫的畜群。

4.3.2 猪

与牛、绵羊和山羊相比，对猪进行血清学检测效果不理想。检测应建立在群体基础上。由于大肠埃希菌 O：157，沙门菌、小肠结肠炎耶尔森菌和其他细菌并发感染，经常导致非特异性凝集并产生交叉反应抗体。虎红平板试验用于筛查大多数血清。不建议对猪使用试管凝集试验。补体结合试验得出的结果与虎红平板试验有可比性。酶联免疫吸附试验在当前所有血清学试验中灵敏度和特异度最高。

无论在猪的个体还是群体水平上，皮内试验都是最可靠的诊断程序。当发现畜群存在感染时，因许多受到感染的牲畜可能尚未被检出，故应整群屠宰。

4.3.3 骆驼、水牛、驯鹿、牦牛

用于牛的血清学试验对这些动物同样适用。补体结合试验检测骆驼血清应当 60 ～ 62℃灭活 30min。依沙吖啶（利凡诺）试验曾被推荐检测水牛血清。由于评价有限，酶联免疫吸附试验尚未广泛用于这些物种，但经标化后，有潜在的用途。

4.3.4 犬

检测牛的诊断方法也可用于检测感染牛种、羊种、猪种布鲁氏菌的犬，酶联免疫吸附试验除外，因为在犬中该试验评价较少。犬种布鲁氏菌感染最可靠的检测方法是病原分离。

因常见持续性菌血症，血培养是有效的方法。血清学检测效果不理想，必须用犬种或绵羊附睾种布鲁氏菌替代光滑布鲁氏菌的表面抗原，并且不能产生交叉反应。酶联免疫吸附试验可能是最有效的方法，但未被广泛应用。

动物布鲁氏菌病诊断要点

- 从流产物、奶或解剖收集的组织中进行布鲁氏菌培养用来提供明确诊断。
- 血清学检测通常是最可行的方法。
- 牛：推荐使用虎红平板试验进行筛查；推荐酶联免疫吸附试验或补体结合试验在个体水平确认感染。通过乳环试验或酶联免疫吸附试验筛查牛奶样本进行监测。
- 绵羊、山羊和猪：个体水平上，没有任何一种可靠的血清学检测能确认感染。血清学检测应在群体水平上使用。同样，皮内试验也用于群体水平的筛查，尤其是未免疫畜群。
- 犬种血清学检测必须使用粗糙型抗原。

5. 人布鲁氏菌病治疗

人布鲁氏菌病治疗的基本要素是保证抗生素的有效性和足够的疗程，应在医生监督下进行治疗，特别是重病患者在条件允许情况下应住院治疗。包括即将自愈的病例，都应尽早进行抗生素治疗。必要时，有并发症者可进行手术治疗。

简单的急性期患者能够很好地配合抗生素治疗，医生应使患者及其家属坚信，通常布鲁氏菌病在临床和细菌学上能够彻底康复。

多种抗布鲁氏菌药物在体外常规药物敏感试验有效，但这与临床疗效没有直接关系。因此，β－内酰胺类抗生素（例如青霉素和头孢菌素）、大环内酯类抗生素（例如红霉素）用于治疗布鲁氏菌病的复发率极高。虽然更新的大环内酯类抗生素（如阿奇霉素和克拉霉素）在体外抗布鲁氏菌效果比红霉素更好，但并未显示出治疗优势，其在治疗中的作用仍有待确定。

5.1 无并发症成年人和 8 岁以上儿童布鲁氏菌病治疗

5.1.1 四环素

长期以来人布鲁氏菌病标准治疗方案是四环素（每 6 小时口服 500mg）治疗至少 6 周。现在多西环素（类似长效四环素）是首选药物，每天 1 ～ 2 次口服，并且胃肠道不良反应比

四环素少，给药剂量为每 12 小时口服 100 mg，6 周为 1 个疗程。

5.1.2 氨基糖苷类

由于四环素或多西环素单独用药的复发率达 10% ～ 20%，故多数官方建议疗程前 2 ～ 3 周将四环素与氨基糖苷类抗生素联合应用。

当氨基糖苷类抗生素与四环素或多西环素联合应用时，首选链霉素（每天 1g）肌内注射 2 ～ 3 周，虽然这两种药物在常规体外药物敏感试验中很难证明能起到协同作用，但是细菌杀灭研究表明，两种药物联合比单独应用更有效。

庆大霉素体外药物敏感试验比链霉素更有效，每日一次给药时，极少产生不良反应。一项研究显示，将庆大霉素以 5mg/（kg • d）的剂量进行静点或肌内注射，与多西环素联合应用 7 ～ 10 天，6 周 1 个疗程，取得了很好的效果，但此类研究有限，尚不能证明该方案疗效超过多西环素 + 链霉素。遗憾的是，尚无多西环素 + 链霉素和多西环素 + 庆大霉素疗效比较的直接研究。目前有些关于使用庆大霉素代替链霉素治疗的研究，但最佳剂量和疗程仍未知。

5.2 首选疗法

布鲁氏菌体外对具有显著脂溶性且能蓄积在真核细胞内的利福平敏感。为了给布鲁氏菌病治疗提供完全口服的疗法，1986 年 WHO 专家委员会推荐，多西环素（每天口服 200mg）+ 利福平（每天口服 600 ～ 900mg）联合治疗 6 周。这种治疗方案与多西环素 + 链霉素治疗无并发症布鲁氏菌病疗效相似，有并发症（如脊柱炎）的病例则需要谨慎处置。多种治疗方案分析显示，多西环素 + 链霉素的方案可能是最有效的。此外，有报道表明，利福平可以增加多西环素的血浆清除率，从而未达到治疗水平，这可能是导致该方案治疗失败的原因。

5.3 次选疗法

氟喹诺酮类：氟喹诺酮类抗生素布鲁氏菌体外敏感试验优于药物萘啶酸。此外，口服后较易吸收，并且在吞噬细胞内浓度较高。虽然有报道喹诺酮类药物的最低杀菌浓度约是最低抑菌浓度的 4 倍，但与细胞内相比，在 pH 相近的其他条件下，发现其缺乏杀菌活性。此外，单独用氟喹诺酮类药物治疗实验动物和人布鲁氏菌病时，复发率很高。因此，氟喹诺酮类药物总是与其他药物联合应用，如多西环素或利福平。

甲氧苄啶 / 磺胺甲噁唑（TMP/SMZ，复方磺胺甲噁唑）：甲氧苄啶 / 磺胺甲噁唑按 1 ： 5 的比例（80mg TMP，400 mg SMZ）抗布鲁氏菌体外药敏试验活性比单独使用更好。虽然初步研究甲氧苄啶 / 磺胺甲噁唑效果较好，但对比试验发现这种疗法有较高的复发率，因此，甲氧苄啶 / 磺胺甲噁唑应与另外一种药物联合使用，如利福平、多西环素或链霉素。

5.4 布鲁氏菌病并发症的治疗

5.4.1 脊柱炎

布鲁氏菌病骨关节并发症常见，发生率达 40% 以上。一些临床表现似乎不需要特殊治疗，如关节炎。与此相反，布鲁氏菌病相关脊柱炎和骨髓炎可能需要长时间治疗，如脊椎增生和硬膜外脓肿，可连续使用多西环素 8 周甚至更长时间。几乎不需要手术引流。

5.4.2 神经性布鲁氏菌病

因为要保持 CSF 中药物的高浓度，所以布鲁氏菌病中枢神经系统并发症的治疗是个特殊问题。氨基糖苷类和四环素不能穿过血脑屏障，所以建议将利福平或复方磺胺甲噁唑添加到多西环素和链霉素的标准治疗方案中。神经性布鲁氏菌病的最佳疗程尚未确定。多数专家建议 6 ～ 8 周或更长，这取决于临床反应。

5.4.3 布鲁氏菌心内膜炎

因布鲁氏菌病死亡的病例少于 1%，导致死亡最常见的并发症是感染性心内膜炎。因为需要在瓣膜赘生物中达到药物杀菌浓度，所以布鲁氏菌心内膜炎的治疗也是特殊的问题。此外，延误诊断会导致瓣膜的进一步损害。故有必要联合使用两种抗菌药物治疗并结合手术更换损坏的瓣膜。多西环素和氨基糖苷类药物的联合应用能快速杀灭布鲁氏菌，利福平和复方磺胺甲噁唑能穿透细胞。推荐延长疗程（至少 8 周），并且在手术置换瓣膜后持续治疗数周。

5.5 孕期布鲁氏菌病治疗

对患布鲁氏菌病的孕妇做出及时诊断和抗菌治疗可以挽救胎儿生命。妊娠期和哺乳期妇女应选择适当的药物治疗。所有药物都能不同程度上穿过胎盘，这表明药物对胎儿有潜在影响。四环素可使胎儿牙齿永久性着色，并且易引起孕妇的肝脂肪坏死和胰腺炎，故妊娠期禁用。氟喹诺酮、利福平和复方磺胺甲噁唑等药物的潜在致畸性未知。有孕妇应用链霉素治疗导致胎毒性的报道，然而没有庆大霉素毒性的报道。因此，对于治疗妊娠期布鲁氏菌病的最佳方案仍未确定。有复方磺胺甲噁唑成功治疗孕妇的报道。也可用利福平至少治疗 45 天，这取决于最终疗效。

5.6 ＜ 8 岁儿童的布鲁氏菌病治疗

对于新生儿和＜ 8 岁儿童布鲁氏菌病的最佳治疗方法还没有明确界定。因为牙齿的色素沉着和影响骨骼发育而禁用四环素。尚没有研究能够证实多西环素比其他四环素药物较少与钙结合且造成的风险较小。因此，氨基糖苷类、复方磺胺甲噁唑和利福平是通常推荐的药物，但厂家不推荐复方磺胺甲噁唑和利福平用于年幼儿童，并且单独使用时复发率高。复

方磺胺甲噁唑［8 ～ 40mg/（kg • d），分 2 次口服］连用 6 周加链霉素［30 mg/（kg • d），每日 1 次肌内注射］连用 3 周或庆大霉素［5 mg/（kg • d），每日 1 次静脉滴注或肌内注射］连用 7 ～ 10d。备选方案包括复方磺胺甲噁唑加利福平［5 mg/（kg • d），口服］）连用 6 周，或利福平加氨基糖苷类药物。上述方案可积累治疗经验，但目前仍无法判定儿童的首选治疗方案。

5.7 暴露后预防

随着活疫苗免疫牛（牛种 S19 和 RB51）、绵羊和山羊（羊种 Rev1）的增加，兽医免疫感染的问题变得更为普遍。疫苗针头扎伤多导致穿刺伤口，但通常被注入疫苗较少。然而仍有潜在感染风险，建议加强伤口局部护理和 6 周的多西环素治疗，必要时注射破伤风类毒素。需注意的是 RB51 对利福平耐药。相比之下，布鲁氏菌飞溅入眼睛（结膜接种）是传播布鲁氏菌病很危险的方式。因此，对于结膜途径等接种事故，建议局部眼部护理和使用一或两种药物治疗 6 周。此外，事故后应尽快检测血清布鲁氏菌抗体，为发病后随访提供基线。

5.8 疫苗与免疫系统刺激药

尚无证据能够证明接种布鲁氏菌疫苗或抗原制剂是有效的，也无证据能够证明使用左旋咪唑等免疫系统调节药治疗人布鲁氏菌病是有效的。对局部并发症应谨慎使用抗生素类药物，要尽量咨询专家。

人布鲁氏菌病治疗要点

- 人布鲁氏菌病治疗的基本要素是保证抗生素的有效性和足够的疗程。
- 无并发症成年人病例和 8 岁及以上儿童病例治疗：多西环素每天 2 次，每次 100mg，持续 6 周 + 链霉素每天 1g，持续 2 ～ 3 周。
- 或：多西环素每次 100mg，每天 2 次，持续 6 周 + 利福平每天 600 ～ 900mg，持续 6 周。

6. 人布鲁氏菌病的预防

人布鲁氏菌病的根本来源是直接或间接暴露于感染的动物或其制品，预防必须基于避免接触。显而易见，消除动物布鲁氏菌病是最好的方法，但发展中国家缺乏所需的经济和社会资源。消除小型反刍动物布鲁氏菌病的技术和社会难题甚至加重了一些发达国家的负担。许多情况下，只能通过个人卫生、采用安全操作准则、保护环境和食品卫生来

最小化疾病的影响和降低感染风险。缺乏安全、有效、广泛应用的人用疫苗，意味着当前的措施对预防人布鲁氏菌病的作用微乎其微。

在工业化国家和非游牧业国家，感染主要来自：①职业暴露；②食用污染食物。

在游牧、迁徙饲养或小型传统农场，感染来源几乎都是直接暴露于患病动物或污染的食物。

在艰苦条件下生活的人群中，尤其在干旱半干旱地区，很难开展初级卫生保健，但实施一些基本的措施能明显降低患布鲁氏菌病的风险。

6.1 职业卫生

直接接触动物及其制品的职业人群感染风险最大，包括农民、饲养员、牧民、屠宰工、肉贩、乳品加工工人、人工授精员、兽医和与牲畜内脏、皮毛等有接触的人群。维修相关建筑和设备的人员也可能面临风险。暴露于污染样本或进行菌体培养的实验室工作人员也是重要人群。无论在诊断还是免疫过程中（例如生产和使用活疫苗），都会带来一些风险。

6.2 个人卫生

与确诊或疑似布鲁氏菌病动物接触时，进行高风险操作的所有人都应该穿着防护衣，包括连体衣或上衣、橡胶或塑料围裙、橡胶手套、靴子以及护目用品（面罩、护目镜、口罩）。处理流产或分娩动物时风险最大，此外，危险行为还包括剪羊毛、检验、免疫、治疗、消毒和清扫污染场所等。

工作场所应常备工作服，使用后应消毒，方法可以是加热消毒（蒸或煮），甲醛熏蒸，消毒液浸泡（聚维酮碘、苯酚、氯胺、次氯酸盐）。应特别注意鞋类消毒，以确保布鲁氏菌不被传播至外界场所或房屋、帐篷中。

理想情况下，操作者应彻底清洗或使用喷淋设施。至少要用 1% 的氯胺溶液（或其他消毒液）洗手，之后用肥皂水和清水冲洗，再涂抹护手霜。切口或划痕等任何表面伤害都应用抗菌药治疗，如碘酒消毒后使用绷带或创可贴。

结膜存在高感染风险，故眼部防护尤为重要。任何感染物进入眼睛，都应远离工作区并在无菌条件下清除。用流水彻底冲洗眼睛后，使用氯霉素或四环素滴眼液或药膏。

环境被严重污染时，呼吸道感染也存在高风险。预防吸入来自流产、分娩、屠宰组织物或干燥粪便的灰尘或气溶胶。应该佩戴能滤除细菌的口罩，定期更换口罩并对其进行化学消毒或湿热消毒。

理论上，应对职员进行定期血清学监测。强烈建议新职员在开始工作前提供血清样本基线。发展为有临床症状的患者都应被及时治疗。＜ 18 岁的年轻人和孕妇不应从事高风险职业。

6.3 农场卫生

当与感染或可能感染动物接触或环境已被患病动物的流产或分娩物、排泄物污染时，农场工人和动物饲养员应穿着防护衣。尤其重要的是，为流产或分娩动物接生时，排出的布鲁氏菌会达到最高水平。

流产胎、胎盘和受污染的垃圾应被收集到密封容器中，妥善焚烧处理。也可以在远离水源的地点用新的生石灰深埋。应使用消毒剂（推荐工作浓度的次氯酸盐、聚维酮碘、苯酚）冲洗流产或分娩的区域。

应将处理污染材料后的农具浸泡在消毒剂（聚维酮碘、苯酚或稀释氢氧化钠）中消毒。

粪便应每日清除并堆积于偏僻区域，自然降解直至安全（可能需要约 1 年的时间）。或者在处理前焚烧或用消毒液浸透。粪水中长期存在感染物，尤其在低温时。添加氰氨化钙或二甲苯可以加速杀灭布鲁氏菌，但至少存放 6 个月。

出入受污染场所的车辆应当驶过盛有消毒剂的浅水槽、浸透消毒剂的草或海绵。感染动物饲养场所清理消毒后，至少 4 周后再重新使用。维修工人（如建筑工、水暖工、电工）禁止进入未经净化场所。

建筑物应防鼠防虫。使用纱窗、诱虫灯和杀虫剂将虫害降至最低。

6.4 游牧或迁徙条件下的布鲁氏菌病预防

即使在一些发达国家，也常在半游牧条件下饲养绵羊和山羊，导致农场自身的卫生措施较难实施。在干旱、半干旱地区，完全游牧条件下，这些措施更难实施，几乎无法遵循卫生操作来预防感染。可通过疾病特点和传播模式的健康教育以降低疾病的影响。上述人群中，大部分成年人已经暴露于布鲁氏菌，可能会有一定程度的免疫力。此时该病对于儿童影响最大，应该避免儿童接触幼畜以及最近流产和分娩的家畜。尽管风俗习惯很难改变，但也应劝阻人们食用生的奶、血、肉或内脏。

此类人群的唯一预防措施是疫苗接种，但目前尚无令人完全满意的疫苗。

6.5 肉品加工厂和炼油厂的卫生防护措施

感染羊种菌的绵羊、山羊和牛，感染猪种菌的猪在屠宰阶段都特别危险。在菌血症期布鲁氏菌普遍存在于组织中。乳腺、子宫、睾丸都可能严重感染。近期流产或分娩的动物也可造成广泛的环境污染。

当解剖病牛的子宫或乳腺时，尤其是处于孕期或哺乳期的病牛，可能释放出大量布鲁氏菌，这给屠宰场工人带来严重风险。建议屠宰前使这些动物停止产奶，若不可行，则焚烧销毁病畜尸体。

如果已知动物感染了布鲁氏菌，应该在特定的屠宰场进行屠宰，这些屠宰场应对员工

进行风险培训且具备相应装备。屠宰工应穿着全套防护服，包括防水工装裤或围裙、靴子、口罩、护目镜或面罩。必须佩戴橡胶手套，同时使用防割手套以防止意外割伤。工作区域内禁止饮食和吸烟。应提供适当的防护衣、消毒工具和个人清洁设施。

如果无特定的屠宰场，每天应在屠宰健康动物之后屠宰感染动物。乳房和生殖器等器官极易被严重感染，应予以销毁。

每天工作结束后必须对场所和设备进行全面清洁和消毒。建议将动物组织和废物置于塑料袋等密封的容器后焚烧处理。

仅限从业人员进入屠宰场，但＜ 18 岁的年轻人和孕妇禁止进入。如有可能，应从血清学证明既往暴露于布鲁氏菌的人中挑选员工。

员工需持续进行医学监测，对有症状的布鲁氏菌病患者应用抗生素治疗。所有员工，尤其是育龄期妇女必须获悉布鲁氏菌病感染相关风险。应该告知员工哪些因素能够影响免疫状态（例如怀孕、免疫抑制药物、肿瘤等）。提高员工认知意识，鼓励其向权威医疗机构咨询，以获得适当的医学建议和指导。应对员工进行安全生产和防控措施的教育培训，这对新员工更加重要。

6.6 实验室安全措施：处理可能含有致病性布鲁氏菌材料的防范措施要求

布鲁氏菌在世界卫生组织第三类风险中，即对相关工作者造成高风险，但对大众是低风险的病原体。布鲁氏菌病实际上最容易造成实验室感染。风险程度不只与病原体的毒力有关（羊种菌和猪种菌对人类最危险），还与处理材料中病原体数量有关。进行血清学或病原学诊断时，血液样本或活检标本中所包含的布鲁氏菌数量不足以导致操作人员出现重大风险，但应在生物安全 2 级实验室中小心处置。通常会在一般医疗机构处理这些可能含致病菌的样本（彩图 8）。然而布鲁氏菌在培养基中生长后，病原体数量增多而危险性增大，因此，必须严格防范，此时需具备 3 级生物安全水平的试验设施和操作规程。处理动物流产物时同样适用。凝集的血液样本风险很小，牛奶样本仅有轻微风险。每克胎膜、胎儿组织和体液可能包含多于 10 亿的布鲁氏菌，在实验室中处理培养物时可能接触到与之相当数量的布鲁氏菌。处理危险物的防范措施如下。对实验室安全措施的全面阐述见世界卫生组织实验室生物安全手册（第 3 版），其中包含许多有价值的实验室生物安全信息和相关参考文献。

6.6.1 实验室操作致病性布鲁氏菌的相关要求

谨慎处理培养物和其他潜在高危材料，如属于生物安全 3 级的胎膜、胎儿组织和体液。每个房间要求只能用一个入口；入口处张贴生物安全警示，禁止未经批准人员进入。理想情况下，为提供气闸房间入口应该有两重门。通风设备应保持房间内压力稍低于周围环境。房间内的空气应可以向外排出，远离进气口和敞开的窗户，否则一定要通过过滤或加热进

行灭菌处理。墙应不可渗透，密封所有窗口以灭虫和熏蒸。应防范啮齿动物和昆虫进入。房间必须正确安装和检测Ⅱ级或Ⅲ级生物安全柜。从安全柜排出的空气，应避免阀门打开时妨碍房间内部以及安全柜内部的风量平衡。房间应有水槽、高压灭菌器和足够的培养箱。靠近出口处必须提供洗手设施。

6.6.2 生物安全柜

见世界卫生组织实验室生物安全手册第 3 版，其中有生物安全柜的使用和选择等信息。

6.6.3 常规预防措施

自有记录以来，布鲁氏菌病作为最常见实验室感染的疾病之一，其实施（使用）合适的生物安全措施（设备）的重要性不能被忽视。每个实验室都应该有书面的设备使用规程（尤其是能产生气溶胶的设备）。消毒设备和污染物、处理加工样本、围堵和清除溢出物以及处理废弃物等规程应清晰简明、易于理解、严格执行。如前所述，生物安全 3 级适用于处理布鲁氏菌培养物和感染的胎膜、胎儿组织和体液。

6.6.4 特定试验流程的预防措施

抗原生产过程中，应使用低毒力菌株，如牛种布鲁氏菌 1119-3[1]，19[1,2] 或 99[2]。此外它们的优点还有生长不需要添加 CO_2。

离心机可以产生危险的气溶胶，尤其是当装有强毒细菌的试管破裂时。不应用玻璃试管盛装强毒细菌，推荐用有紧实螺旋帽盖的树脂试管替代玻璃试管。如果必须离心强毒细菌，必须在生物安全柜中加样和移样。连续流式离心机不应用于强毒细菌。电子搅拌器、超声波发生器等类似仪器应在安全柜中使用。

除严重污染材料外，细菌学诊断的首选方法是直接培养，而不是接种实验室动物。如果必须进行潜在危险物质的动物接种，只能用锁定针头的注射器。处理前不应再将针头保护帽盖回，操作者应穿戴防护衣和口罩。

6.6.5 卫生和医学监测

所有操作强毒布鲁氏菌者都应受到密切的临床和血清学监测。在某些国家，要求对有特殊风险的人员提供预防接种，但当前疫苗效力不佳并可能导致不良反应。

6.7 食源性布鲁氏菌病的预防

由于一般人群不与动物直接接触，所以布鲁氏菌病最大的潜在传播途径是通过食用未经高温消毒的奶及奶制品。肉也是重要的传染源，尤其是在某些民俗习惯中，人们喜欢食用生的或未煮熟的肉类产品。

6.7.1 奶和奶制品

患病牛、绵羊、山羊、水牛、牦牛、骆驼和驯鹿的奶中可包含大量布鲁氏菌。因食用量大或被添加到其他食品中（例如奶油或奶酪），从而带来特别严重的危害。鲜奶制作的软

奶酪可含有大量的布鲁氏菌。建议不要制备这些未经处理的奶制品。若很难改变当地饮食习惯，奶酪应储存6个月后方可上市销售。硬奶酪存在丙酸和乳酸发酵，故酸化后通常危险较小。如果将制作奶酪剩余的未经灭菌的乳清喂给动物可以造成感染。盛装奶和奶制品的容器使用前应消毒，否则会造成交叉污染。

用感染牛胃制备的凝乳酵素制作奶酪，这也会成为传染源。

黄油、酸奶、酸奶酪的酸化过程都能大大减少布鲁氏菌含量，但酸度必须低于 pH 3.5，以彻底杀灭布鲁氏菌。

污染奶制作的冰淇淋特别危险，尤其不同奶源的奶混合制作的冰淇淋。所有用于制作冰淇淋的奶和奶油均应加热处理。

煮沸或巴氏消毒会杀灭奶中的布鲁氏菌。理想情况下，所有出现布鲁氏菌病地区的奶制品都应巴氏消毒。若不具备巴氏消毒条件，应加热到最低 80 ～ 85℃并维持几分钟或煮沸。这适用于人食用的所有奶，不论饮用还是用于制作其他食物。

6.7.2 肉类

肌肉组织包含布鲁氏菌浓度极低，若食用前储存得当，布鲁氏菌的数量还将进一步降低。肾、肝、脾、乳房和睾丸可含有更多的布鲁氏菌。如果彻底煮熟，他们不会出现严重的风险。但在某些民俗习惯中，人们喜欢吃生的或未煮熟的肉、直接食用新鲜血液、将新鲜血液与奶混合食用，这些做法应当被劝阻。

在处理感染的肉和内脏时，若没有适当的卫生防范措施，会导致其他食品的污染。烘干、腌制、熏制等方法对于杀灭布鲁氏菌均不可靠。同样，在冷藏箱或低温冰箱中，布鲁氏菌可正常生存。强烈建议在食用前彻底加热所有的肉制品。

6.8 疫苗 *

尚无预防人布鲁氏菌病的安全有效的疫苗，但在苏联和中国，免疫在预防布鲁氏菌病方面发挥了重要作用。在严重感染地区，两种减毒活疫苗被广泛使用。

1952 年起，苏联就开始使用牛种 19-BA 疫苗。采取皮上划痕的方法，使用剂量 1×10^9。保护效果 1 年以上，但最大功效在免疫后 5 ～ 6 个月。因此，通常在畜间发病高峰之前免疫。总的来说，在健康成年人中给予皮上划痕接种耐受良好。76% 的接种者发生充血和硬结的局部反应。3% ～ 7% 出现以头痛、嗜睡、低热为特征的全身反应。有证据显示，免疫之前曾暴露于布鲁氏菌更易出现全身反应。

流行病学研究显示，在高风险地区疫苗有助于降低发病率，能减少急性布鲁氏菌病报告病例 5 ～ 11 倍。但疫苗易引发过敏，尤其是多次或超剂量接种的情况下，更容易引起过敏。

中国曾使用牛种 104M 活疫苗减毒株。采取皮上划痕的方法，使用剂量为（7 ～ 10）$\times10^9$。

* 见陆生动物诊断试验与免疫手册，世界动物卫生组织，第 5 版。

该菌株与牛种 19-BA 相比，毒力更强。皮下注射可引起严重反应。使用适应证类似于牛种 19-BA。要注意避免接种已自然感染的人群和重复接种。

当前没有质量控制程序符合国际标准的厂商来生产这些活疫苗，因此，其有效性和使用受到很大的限制。近年来更加强了亚细胞片段的非活体疫苗的研发。以下两种疫苗已经被广泛研究。

法国研制出肽聚糖片段（PI）疫苗，该疫苗从羊种 M15 布鲁氏菌萃取的不溶解于苯酚的脂质残渣中获得。随后又从牛种 S19 布鲁氏菌中提取到 PI。在职业暴露人群，特别是实验室工作人员中一直使用。1mg 分 2 次皮下注射，中间间隔 2 周。该疫苗无毒，极少引发全身反应，致敏性弱，不会导致严重过敏现象。但有报道称该疫苗可促进淋巴细胞增殖来应答布鲁氏菌抗原，但这和免疫力有关。有报道保护作用能长达 2 年。尽管近 20 年里约有 2000 人应用了该疫苗，但尚无临床对照试验证明其有效性。目前该疫苗尚未大量生产。

在俄罗斯，另外一种亚细胞片段“布鲁氏菌化学疫苗”（BCV）被研制出来。该疫苗使用 0.1M 醋酸从牛种布鲁氏菌 19-BA 的细胞壁中提取，并且包含蛋白 - 多糖复合物。用量为 1mg 肌内注射，仅有轻微局部刺激和全身反应。即便先前曾经暴露，也不产生严重的过敏反应。保护性堪比 19-BA 活疫苗株。哈萨克斯坦研究显示，75 000 例接种对象中 BCV 疫苗和活疫苗保护效果分别为 79.6% 和 76.6%。BCV 疫苗与减毒活疫苗相比引起的皮肤过敏降低 50% ～ 75%。1 年后可重复使用并无严重过敏的风险。该疫苗值得进一步广泛评估。

当前其他疫苗正在研发中，包括有明显减毒突变的活疫苗菌株和 LPS- 蛋白结合疫苗。

6.9 公共卫生方面

从公共卫生的角度来看，布鲁氏菌病的主要来源与食品或接触感染动物有关，包括职业或娱乐性的接触。除了要适当控制通过血液和器官传播外，人际传播不是重要问题。当感染动物经过人口密集区（如去集市的路上）导致环境污染时，空气传播或直接接触污染物传播的问题比较严重。应采取适当措施解决这些问题。关键方法是对人群进行教育，尤其是直接从事畜牧业和食品加工行业的人群。

控制规划的所有措施应进行充分的设计和有效的实施。公共卫生、兽医机构及其他相关部门密切协作是实现控制目标的基础。

6.9.1 公众健康教育

食品安全是保障人类健康的主要支柱之一。人被布鲁氏菌感染主要通过错误地制备和（或）保存动物源性食品。

当前有足够系统的科学知识、技术和程序来实现食品的安全制备和消费。但比较缺乏运输、加工、保藏方面的食品安全知识，特别是在发展中国家。而且，食品加工厂主对这方面常不感兴趣，甚至不遵守食品安全条例（见附录 A）。

在世界上许多地区，包括布鲁氏菌病在内的食源性疾病发病率相当高。主要对幼儿和老年人产生重大影响。

食源性疾病不仅使人类遭受病痛的折磨，还造成重大的经济损失，包括收入和人力资源损失、医疗保健费用损失、加工不当和腐败导致的食物损失。因此，布鲁氏菌病防治计划或框架应包括公众健康教育，甚至可将布鲁氏菌病公众健康教育作为一个独立的教育活动。健康教育是一项艰难和非常复杂的任务，必须具体考量社区的文化、信仰、传统、教育水平、社会地位、职业、年龄等具体因素，所以健康教育项目应针对特定社会人群，其中包括可能没有完全认识到该问题的医生、兽医和农民。健康教育不仅需要直接指导具体防治措施，而且应强调提高社区卫生和自身健康的责任感。主要目标是使每个人明确自身的问题和需求，并了解可以使用哪些自身资源和外部支持来解决所遇到的问题。只有在深入了解社会和环境背景的情况下，健康教育才能达到最佳效果。

健康教育原理和方法参照附录 B。

6.9.2 社区参与

如果没有社区参与，卫生规划则不完整而且在当地无法执行，单独的法律、条例和兽医政策也不会达到预期效果。社区的健康教育需要学校、工作场所、全体人群共同参与。

首先，自立性和社会意识越强，个人和家庭越会勇于承担责任，来保护家畜和自身防止来自食物、环境等直接传播的疾病危害。社区教育相关课程的重点应是如何改善自身健康状况。

其次，社区成员应参与到影响其个人的卫生规划设计当中，因为他们了解本地的社会结构、状况、资源和需求。

再次，社区成员应积极参与本社区卫生规划的实施。他们有重要的优势条件，包括讲本地方言、知道如何与居民沟通、如何与家畜接触以及更易被当地群众认可。社区组织参与健康教育活动参考附录 C。

促进社区参与并无单一模式。由于社会、文化、政治和经济因素影响，社区参与人兽共患病控制项目的程度会因环境不同而不同。合适的指导原则能够适用于不同的环境并贯彻始终。尤其应使流行地区的公众认识到疾病对健康的危害、人兽共患病和食源性疾病造成的经济损失。尽可能充分利用大众传媒。所有能在社区间传递信息的手段都应该被使用，小组讨论则是一个有效的方法。这些讨论中，卫生工作者（教育者）建议一些具体行动，例如，讨论后很快组建工作委员会，经证明这些委员会在控制项目早期十分有益。

6.9.3 卫生工作者和学校教师的公众健康教育培训

健康教育者应尽可能来自本地社区。动物传染病和食源性疾病防控项目涉及的每个人都必须开展公众健康教育。因此，工作人员的培训至关重要。应制定工作人员培训计划，向相关专家咨询，以选择适合本地条件和不同项目阶段的教育方法和材料。

大多数健康教育工作者都已经是专业人员或本领域的专家，因此，应大幅消减培训班

的理论课程，最好采取能让健康教育工作者积极参与的教学方法，如提出并解决问题、案例研究、小组合作和角色扮演。

对于大多数人来说，学校是最重要的学习场所。对儿童的科学教育不仅影响其自身，还影响到他们的下一代。儿童主要通过两个渠道受到影响，即父母和老师。他们应培养儿童健康的生活方式和生活习惯。值得注意的是，许多流行区的学龄儿童和年轻人不能定期上学，因此，健康教育应走出学校向包括高危人群的弱势群体扩展。

工作场所是开展健康教育的另一重要地点。应教导食品行业工作者、负责人和食品加工设备所有者有关食源性疾病的潜在危险因素和预防措施。

更多健康教育项目策略见附录 C。

人布鲁氏菌病预防要点

- 人布鲁氏菌病预防基于职业卫生和食品卫生。
- 通常不推荐人接种疫苗。
- 所有奶制品的制备都应食用加热处理后的奶。
- 避免食用生奶或生奶制品。
- 肉需要充分煮熟。
- 实验室工作者应采取特殊预防措施。
- 医生和卫生工作者应意识到感染布鲁氏菌病的可能性。
- 公众健康教育应强调职业卫生和食品卫生。

7. 动物布鲁氏菌病的预防、控制与消除

预防布鲁氏菌病传入畜群与控制已感染畜群的目的一致，都是保障经济效益和公众健康。

布鲁氏菌病是一种在人与动物之间有很强相关性的动物传染病，虽然巴氏灭菌和健康教育等公共卫生措施取得了不同程度的成功，但主要还是兽医负责运用畜牧学和流行病学原理来控制布鲁氏菌病。预防和控制布鲁氏菌病的跨部门协作策略见附录 G*。

* 更多信息见陆生动物卫生法典（世界动物卫生组织，第 14 版）中的动物及其制品的国际贸易卫生标准

7.1 预防

预防比控制和消除布鲁氏菌病更经济实用。布鲁氏菌病的预防措施如下。

- 仔细选择换代牲畜。无论购买还是从现有畜群中繁殖，换代牲畜都应当来自无布鲁氏菌的牛群或羊群。除非已知换代牲畜来自无布鲁氏菌病地区，否则购买前必须进行检疫。
- 新购入牲畜至少隔离 30 天，混群前必须再次进行血清学检验。
- 防止与患病或状态未知的畜群接触或混群。
- 实验室检测可用于确定流产、早产以及其他临床症状的病因。可疑的动物应当隔离至诊断明确。
- 对牛群和羊群进行监测，如牛的周期性乳环试验（每年至少 4 次）、通过简单血清学筛查（虎红平板凝集试验）检测屠宰牲畜。
- 合理处置胎盘或死胎（焚烧或掩埋）。彻底消毒受污染地区。
- 与公共卫生部门合作调查人间病例。可以通过调查人间病例来发现羊种菌引起的动物布鲁氏菌病。

7.2 控制

动物控制规划的目的是降低疾病对人类健康和经济的影响，而不是消除布鲁氏菌病，这意味着畜群中可残存一定程度的感染，但此感染水平需在可接受范围内。畜群感染达到“可接受水平”后，控制规划仍需持续进行，以使布鲁氏菌病不再复燃。在许多国家，布鲁氏菌病的控制方法是依靠政府监管或立法，有些国家则不然，因此患病畜群的管理方式差别很大。然而下列原则都是通用的：①减少布鲁氏菌的暴露；②增加畜群抵抗力。这些原则可以被进一步分成：检疫、隔离、屠宰、卫生措施、动物运输管理和免疫。

7.2.1 检疫、隔离或屠宰

动物个体水平上没有能确诊布鲁氏菌病的体征，畜群暴发流产通常是明确的感染指标。因此，通常用血清学（有时是变态反应性）检测鉴别被感染动物。细菌学检测常用于确证检测结果或流行病学研究。

决定屠宰阳性畜前，要考虑管理和流行等因素。多数情况下，若牛、羊群发病率很低（如 2%），阳性畜的检疫和屠宰只能降低畜间发病率。若免疫除阳性畜外的所有牲畜，那么保留阳性畜的危险相对较小，但这只能作为最后的手段。必须隔离阳性畜，尤其在分娩期间和分娩后。

屠宰阳性畜不仅昂贵，还需要所有者的合作和必要的补偿。此外，与牛相比，诊断绵羊和山羊患病的可靠性较低，故检疫和屠宰不大可能成功控制绵羊和山羊布鲁氏菌病。如果未免疫除病牛外的全部牛，此时检疫和屠宰也不一定能取得成功，尤其在大型牛群中。必须

重复对牛、羊群检疫，以进一步降低布鲁氏菌病发病率和确认是否已消除布鲁氏菌病。

7.2.2 卫生措施

控制布鲁氏菌病卫生措施的目标是减少易感动物暴露于患病动物及其排泄物、组织等，这是疾病控制中的常用方法。饲养方式（如牛、羊的混养）、交易模式、流行程度、设施类型以及动物所有者的配合程度等影响因素将决定是否能够成功控制布鲁氏菌病。若牲畜所有者对隔离临产动物等布鲁氏菌病防治知识缺乏了解，则很难或不可能实施卫生措施。

目前尚未普遍使用抗生素治疗被感染或潜在暴露动物，且不能将抗生素治疗作为控制布鲁氏菌病的方法。有研究显示，当牛、羊群被治疗后，可快速降低布鲁氏菌病发病率，但这在现实中难以实施。可对有特殊饲养价值的动物进行治疗，但是由于结果不明确而不被推荐。

7.2.3 动物运输管理

动物运输管理可被视为卫生措施的一个方面。然而，其本质上是限制布鲁氏菌的扩散。动物个体应该被打上标牌、文身或耳标。禁止从感染地区到其他地区未经授权的销售或运输。同样，进口到清洁地区的动物必须来自无布鲁氏菌病地区，牛、羊群无布鲁氏菌病史，且出示近期布鲁氏菌病检疫阴性证明。

实践中，控制游牧或半游牧的骆驼和小型反刍动物比控制密集饲养的肉牛和奶牛更有难度。牛、羊群所有者习惯于穿越边境的季节性迁徙。

7.2.4 免疫

人们普遍认为给动物免疫是预防和控制动物布鲁氏菌病最成功的方法，而理想的疫苗并不存在，已证明针对绵羊和山羊的羊种 Rev 1 和牛种 S19 减毒疫苗株优于其他疫苗。美国和一些拉丁美洲国家已应用不产生凝集素的 RB51 疫苗，并取得了令人满意的结果。疫苗的来源和质量要求十分严格，尤其是 Rev 1，剂量和使用方法的改变都能影响结果。因此，当其他控制措施均失败时，只能推荐对整个牛、羊群进行免疫。使用时，被免疫动物必须进行永久标志并不断监控由疫苗造成的流产。必须从畜群中清除血清学检测阳性的病畜和病原携带畜。

为了使接种后产生的可能混淆诊断试验的抗体刺激最小和避免疫苗造成流产，推荐只在未成熟的雌性动物中使用 S19 和 Rev 1 疫苗。现场和实验室研究显示，使用这些疫苗对牛群和羊群结膜免疫是实用和有效的。快速的群体免疫是成熟的且应用成本最小。低接种剂量会导致抗体滴度产生较低且很快消退。目前已研发出用于区分抗体种类的一些诊断试验，其中补体结合试验和酶联免疫吸附试验被广泛应用。

免疫会避免动物发病和减少患病动物排泄病原体的数量，此外，动物所有者更易接受，因为他们已经习惯了这种方式。在许多国家，免疫是控制动物布鲁氏菌病唯一经济可行的

方法。

越发频繁的动物贸易和大规模饲养已经成为全球性的发展趋势，然而资源是有限的，这给许多国家的布鲁氏菌病防控工作带来了极大的困难。应评估疫情监测和暴发调查等动物布鲁氏菌病的防控工作。评估内容包括标准的病例定义和实验室诊断，但病例定义和实验室诊断应具有一定的灵活性，当出现新信息时，可以被重新修订。

7.3 根除

根除意味着国家或地区（即掌握动物健康状态的国家的一部分）中病原体的消除。无论是在某一个地域还是整个人群中想要达到根除疫情的效果都需要有组织的极大努力。根除概念上不同于控制：它以卫生措施和完全不同于控制项目的实践活动为基础，即便控制项目计划和实施得再好，也不一定能达到根除的效果。

实验室支持的有效监测系统的实施，决策者、农民和利益相关者对于根除布鲁氏菌病的理解和分享，这些都是根除项目成功的关键因素。保持畜群免于感染，必须实施隔离病原的预防措施，并且人畜分开。

上述的预防控制策略可用于根除布鲁氏菌病，然而他们并不互相排斥，反而可将他们放在一个流程图中，见图 1。

长远来看，根除项目通常比控制项目更有经济优势，然而，现实中却往往无法发挥这一优势。根除项目需要大量的人力、财力资源支持，这样的资源是很难具备的，即便具备这些条件，其投入与回报之间的时间跨度也往往超出可承受的范围。在进行足够的流行病学监测前提下，成本效益与成本效果分析可以给制定控制策略提供参考。毫无疑问，若缺乏用来支撑技术和政治决策的流行病学监测系统，就无法实现疫情的控制和根除。

动物布鲁氏菌病防控和消除的要点

- 动物布鲁氏菌病最好的预防措施是加强畜群的管理和卫生措施。
- 免疫对预防和控制感染都非常实用。
- 推荐用 S19 和 RB51 预防牛布鲁氏菌病。
- 推荐用 Rev 1 预防绵羊和山羊布鲁氏菌病。
- 重度暴露时疫苗效果可能受限。
- 预防控制规划需要社会各界的有效合作。
- 控制规划必须有妥善的计划、完备的协调机制和足够的资源。
- 宣传和教育项目对于确保各级社区合作必不可少。
- 只能通过检疫、屠宰结合有效预防措施和动物运输管理来消除布鲁氏菌病。

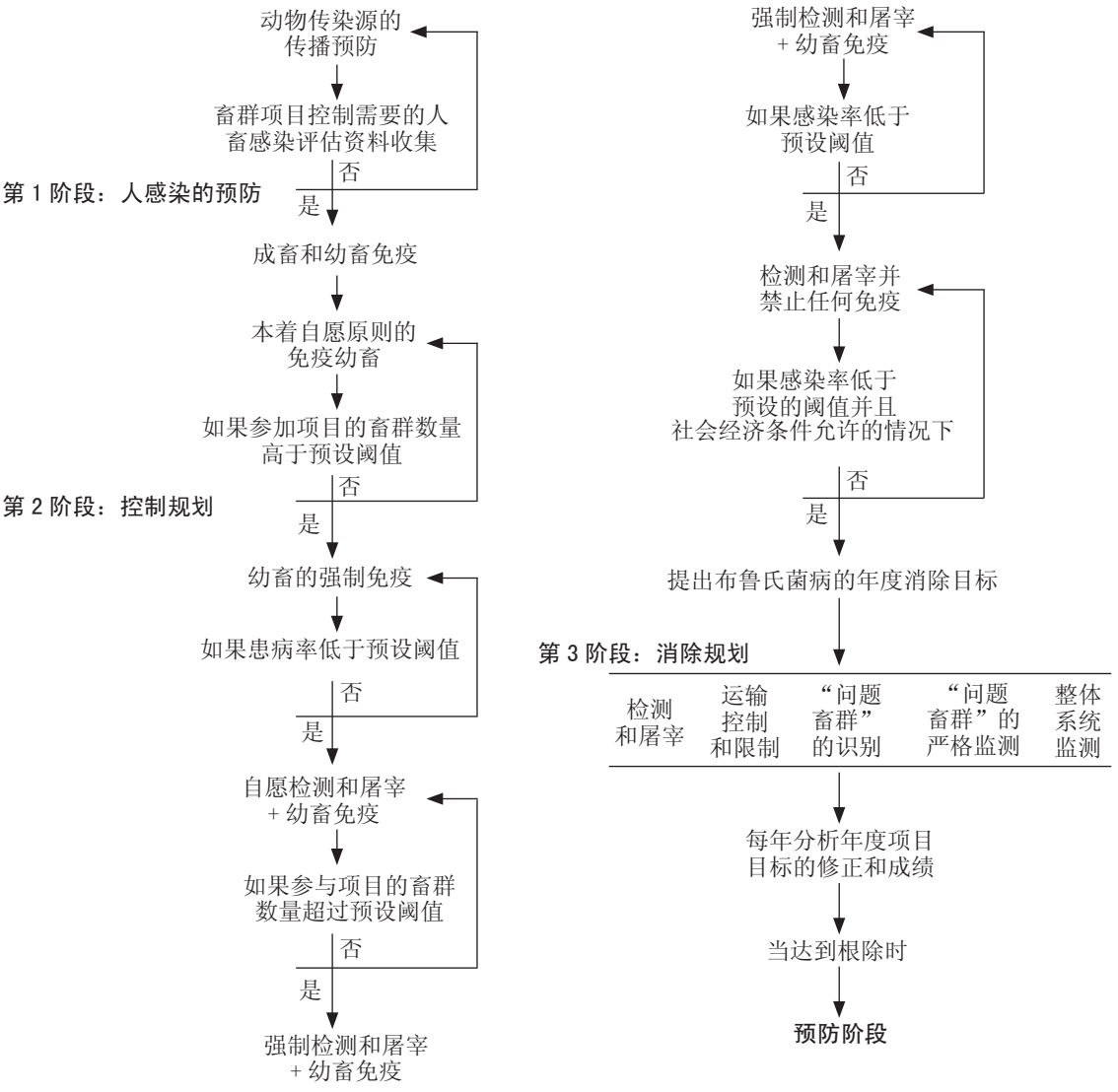

图 1　实现消除布鲁氏菌病的步骤

来源：地中海人兽共患病控制项目，项目国家的人畜流行病学监测第三次研讨会议报告，大马士革，叙利亚共和国，4 ～ 5 日，5 月，1998（http://www.mzcp-zoonses.gr/pdfen/Brucellosis.pdf,acessedJanuary 2007）

8. 监测

监测包括系统地收集、整理、分析和解释特定疾病或症状的数据，并为采取相关行动而及时将该数据传递给有需要的人，以便采取相应措施。监测系统的主要目的是判断应对疾病时是否需要立即或长期采取行动；通过分析数据、判断优先顺序和成本效益、设计备选方案，最终优化资源使用。基于卫生和畜牧部门合作的布鲁氏菌病监测系统，是控制和消除布鲁氏菌病规划的前提。布鲁氏菌病监测系统必须为防治策略服务，即预防、控制或消除人、畜间布病疫情。此外，饲养方式、交易模式、畜牧部门能力等因素也决定了监测系统的类型。

监测方案是统一的整体，应由有着共同目标的机构、设施、活动内容以及方法等组成。

信息的收集与管理成本较高，所以通过最方便的日常活动，收集监测所必需的信息即可。可以用投入、处理和分析、产出来描述监测方案。

监测方案包括主动或被动地收集数据。被动收集信息是将当前活动产生的结果数据供给监测系统，其主要来源是：周边公共卫生服务，周边兽医服务，医院、公共卫生实验室，兽医实验室，边境卫生服务，边境兽医服务。数据的其他来源包括诊所、内科医生、兽医和大学。实验室是人兽共患病数据的主要来源之一，由于群体中样本收集的偏差，实验室数据不能代表群体的清洁状态。然而，实验室起着提供信息的主要作用，若没有实验室，将不能对动物健康状况、预防和控制效果进行监测。

数据的主动收集包括主动搜索和专项收集。主动收集适用于专项调查、被动收集的效果评价、突发事件时开展试验评估。当卫生和畜牧部门没有良好的基础设施时，专项调查是收集监测数据的唯一方法。

分析监测数据的目的是识别、量化卫生服务需求和评估卫生服务产出。需要确定监测指标来追踪并掌握进度。

监测系统的产出通常是卫生状况、资源利用及结果的技术报告，报告要针对不同的对象：外部数据收集者、内部和中心技术人员、决策者等。

8.1 人间监测

有效监测的关键是病例定义，包括可以实施的临床和（或）实验室标准。疑似、可能、确诊病例应分类报告。人间布鲁氏菌病监测推荐标准见附录 H。

医生、诊所和医院进行的病例监测可以揭示人群中存在疾病。不足之处在于，因为布鲁氏菌病报告病例较少，故不太可能给出一个明确定量的发病率指标。轻症病例易被误诊或漏报。

在高危人群中，或在献血者、孕妇等其他进行检测的人群中，临床和血清学调查可以主动收集监测资料。在住院患者、新兵、学生中也可进行调查。由于布鲁氏菌病没有典型

的临床症状，这些监测必须依靠血清学试验。当出现交叉反应时，需要谨慎解释检测结果，包括可能引起假阳性反应的沙门菌 O：30，大肠埃希菌 O：157，小肠结肠炎耶尔森菌 O：9。筛查试验后（如虎红平板凝集试验），至少对一部分样本进行更精确的试验（如 IgG 或 IgA 酶联免疫吸附试验）来支持筛查结果。

细菌学筛查用于人间监测是不实际的，但从患者体内分离培养出的布鲁氏菌应该被鉴定到生物“型”的水平，以追踪可能引起暴发的动物传染源。

在一些国家皮肤变态反应试验被广泛应用于流行病学研究。这比血清学检测更难证明是否患病，充其量只能说明有过暴露史。如果标准化不严格或使用未经纯化的布鲁氏菌，则皮肤变态反应试验结果变化很大。皮肤变态反应试验还会干扰后续的血清学抗体检测，然而，在缺乏实验室设备导致其他检测方法无法实施的地区，皮肤变态反应试验可给出有效的人群暴露水平指标。

8.2 畜间监测

对于人布鲁氏菌病，关键因素是病例定义。由于动物缺乏特异临床表现，为定义动物布鲁氏菌病，实验室检测不可或缺。公共卫生监测的参考单位是患者个体或暴发中的病例，动物监测参考单位是被感染的牛群或羊群，而不是个体的动物。

畜间布鲁氏菌病监测系统数据来源于实验室诊断结果、暴发或个案调查、屠宰场或牲畜市场检测或专门委托当地（国家）的调查。这些数据可以查明该地区牛群或羊群的流行程度，确定被感染牛群或羊群的患病率和发病率。发病率的重要意义是对动物布鲁氏菌病控制和消除效果进行评估。

下列方法可用来主动和被动收集动物布鲁氏菌病资料。不同物种调查的推荐程序见表 4。

表 4　布鲁氏菌病流行病学评估的调查程序

动物		调查程序
牛	奶牛	用乳环试验识别感染的牛群和确认各地区感染牛群患病率。阳性牛群血液样本确认感染牛群中的阳性率
		阳性牛群的牛奶细菌培养用来支持血清学数据并鉴定布鲁氏菌种型
		对流产物进行细菌培养
	肉牛	对待屠宰繁殖期母牛进行血清学检测，随后确认感染农场
		对来自感染农场的血液样本进行检测
		对流产物和淋巴结进行细菌培养
		对农场、市场和马戏团的活畜血清学调查
绵羊、山羊		对屠宰场采集的血液进行血清学检测，随后确认感染的羊群
		对疑似被感染羊群进行血清学检测
		对选定羊群进行皮变试验，然后对阳性羊进行血液检测
		对流产物、淋巴结和阳性羊群的奶样本进行细菌培养

（续　表）

动物	调查程序
猪	对屠宰场采集的血液进行血清学检测，包括非繁殖母猪和阉割公猪，随后甄别感染动物来源 对感染猪群进行血清学检测 对屠宰场收集的淋巴结进行细菌培养 对流产物进行细菌培养
野生动物与家畜接触	对捕（猎）获动物进行血清学检测，病原体分离与鉴定

来源：世界卫生组织、世界粮农组织布鲁氏菌病专家联合委员会。第六报道，技术报告系列，No 740，世界卫生组织，日内瓦，1986

- 区域检测（例如普查）——某区域内所有牛群或羊群的系统检测。
- 选择性畜群检测——高风险畜群的检测，如：与感染畜群邻近或混群的畜群。
- 流行病学调查——追踪新入群牲畜的来源，包括搜索布鲁氏菌病人间病例。这些有时是动物患病的第一手证据，尤其对于羊种布鲁氏菌感染。
- 调查——随机选择畜群检测以确定一个地区的初始患病率，或用以监测发病率。
- 屠宰厂或市场的牲畜检测——可以对阳性牲畜进行初步监测。检测效力随着畜群发病率降低而变小。该监测方式用以判断早期流行。
- 乳环试验检测——广泛用于确定奶牛群布鲁氏菌病现患率和搜索其他感染畜群。每年至少检测散装奶（合成品）3～4 次，并检测乳环试验阳性牛群中的奶牛个体。该检测很敏感，容易出现假阳性，尤其在幼畜群或 S19 疫苗免疫后的畜群。
- 流产调查——某些国家，发生动物流产必须报告给负责疾病控制的部门。

对公牛的监测，可以指示牛群中的感染情况，可以使工作量大大降低。

监测的效力和效果取决于牲畜所有者提供信息的可靠程度和配合程度以及诊断的能力和有效性。

人兽布鲁氏菌病监测要点

- 为了掌握布鲁氏菌病疫情和防控程序的有效性，必须进行持续监测。
- 有效监测的关键是病例定义、监测数据的报告、分析和传播。
- 必须根据控制策略设计监测方案。
- 人间病例可以是畜间疫情的第一指征。

9. 部门合作

上述章节中已经强调了布鲁氏菌病的人兽共患性，这意味着人间布鲁氏菌病的有效预防只能靠消除动物传染源，需要公共卫生和兽医部门在多方面紧密配合。部门合作只是建立有效控制项目的第一步，若要取得成功，还需要群众和政府等社会各界的参与和支持。群众应知晓防范措施，达到自我保护和提高自身状况的目的，政府应提供防控项目所需的资源。在这个架构中，卫生和兽医部门有责任提供专业知识。他们将负责诊断、治疗、监测和执行预防控制措施，还需提供必要的职业相关和社区健康教育信息。部门之间应进行最大程度上的互动与合作，具体处理方法见附录 C。

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附录 A　食品安全的 5 个要点 *

一、保持洁净

1. 制备食品之前和过程中常洗手。
2. 便后洗手。
3. 清洗和消毒用于制备食品的所有设备。
4. 阻止昆虫和其他动物靠近厨房和食品。

二、生熟分开

1. 将肉、禽、海鲜与其他食品分开。
2. 单独使用加工生食品的设备、刀和菜板等工具。
3. 将生、熟食品分开储存在容器中，避免直接接触。

三、彻底烹饪食品

1. 将食品彻底煮熟，尤其是肉、禽、蛋和海鲜。
2. 将汤等食品煮沸，确保加热温度达到 70℃。肉类和禽类的汁水要变清，而不是淡红色的。理想情况下，需使用温度计。
3. 再次加热食品时要彻底。

四、保持食品处于安全温度

1. 室温储存食品不得超过 2h。
2. 快速冷藏加工好的和易腐败的食品（冷藏温度低于 5℃）。
3. 食用前将煮熟的食品加热至滚烫（温度高于 60℃）。
4. 即使在冰箱中，熟食也不宜储存过长时间。
5. 不在室温条件下解冻食品。

五、用安全的水和生的食材

1. 使用安全或处置后确保安全的水。
2. 选择新鲜、卫生的食材。
3. 选择加工过的食品，如巴氏灭菌后的牛奶。
4. 食用水果和蔬菜需清洗，尤其生食时。
5. 不使用过期食品。

* 预防食源性疾病：保证食品安全的 5 个关键常识。日内瓦，瑞士，世界卫生组织，食品安全部，人兽共患和食源性疾病 (http://www.who.int/foodsafety/consumer/5keys/en/index.html, accessed January 2007)

附录 B　公众健康教育方法

教材、教具的内容、形式以及沟通方式，都必须适合目标人群，同时也要符合卫生活动。

世界上不同布鲁氏菌病流行地区的大部分目标人群未接受过教育或仅部分受到过教育，这种情况下，书面文字、小册子和报纸的教育价值较低，幸运的是，广播和电视在许多国家正被广泛使用，甚至牧民还能使用便携收音机。这些大众媒体可以被运用到健康教育中，有效且有趣地将健康教育内容呈现出来。

小型的群组讨论和讲座是非常有用的沟通方式，随后可以成立活动委员会甚至收集诊断样本或免疫接种。将视听教具与群组讨论和讲座结合使用能发挥巨大的优势。此外，海报和照片墙可用在工作场所，提醒处置被感染牲畜或制品过程中需要采取防范措施的工人。教育活动中争取社区领导的支持非常重要。

选择和准备教材的内容时，必须适合教育活动，同时也要适合目标群众的认知、信念和行为。本章其余部分可以当作是每个教育交流的主题，但每个主题必须被分解成简单和容易理解的部分。教育者在健康教育过程中要考虑到民间信仰，尽管有些民间信仰可能很荒谬，但如果忽视这一点，教育活动将会遇到来自群众的阻力，应该用温和的方式改正错误的观念。教材中应充分体现控制布鲁氏菌病带来的预期经济效益。

许多动物所有者和患者不喜欢抽取血液样本用于诊断或其他目的，一些人也不喜欢采血或注射，卫生活动会给他们带来对疼痛和损伤或真或假的恐惧，许多农民由于害怕当前和未来需要的费用而不合作，其他人拒绝合作仅仅由于对健康教育的不了解。健康教育者在准备健康教育工具和材料时必须充分考虑到这些因素。

[来源：S.S. Elberg. 人布鲁氏菌病诊断治疗和预防指南 (unpublished document VPH/81.31. Rev.1). 世界卫生组织，日内瓦，瑞士，1981.]

附录 C　社区组织参与的公共健康教育团体

下列团体在多数社区发挥重要作用

1. 当地的卫生和兽医机构。这些机构中的服务人员不仅是社区项目的参与者，同时还是教育者和推动者。

2. 当地卫生委员会和社区卫生工作者。他们对社区服务的推动和社区教育工作都至关重要。

3. 当地宗教团体。在许多国家，引领人们的态度和行为，对卫生规划的支持至关重要。他们经常为社区活动提供会议大厅、视听设备、社区通信网络等宝贵设施。

4. 当地民间团体。致力于改善社区，集合社区领导并提供人员和资金等资源大力扶持社区活动。

5. 当地学校和成人教育团体。他们身处社区内，可以发动全部家庭，并为小组会议提供设施和资源，吸引社区中值得尊敬且接受过教育的人，在卫生规划中发挥重要作用。

6. 当地传统医学从业者、助产士。通常受到社区的大部分人群尊重，应尽可能地使他们积极参与到卫生项目中。

7. 当地警方或军事单位。通常希望积极地参与社会服务，这些组织必须被告知并参与社区的所有项目。

在农村和城市，社区团体在卫生项目规划和实施上都是十分重要的，他们提供适合当地条件的资源，能够克服困难、以极小的代价执行任务。必须使其了解实现项目目标的方法和自身在教育中所扮演的角色。

（来源：细菌性和病毒性人兽共患病，技术报告丛书 682，世界卫生组织，日内瓦，瑞士，1992）

附录 D　布鲁氏菌噬菌体、氧化代谢、种型特征表

表 D-1　布鲁氏菌种型特征区分

种	型	CO_2 需要	H_2S 产生	尿素酶	硫堇	碱性品红	1/50　000（20mg/ml）出现生长			单一血清凝集	首选自然宿主(1)
							A	M	R		
羊种	1	−	−	+	+	+	−	+	−	绵羊、山羊	
	2	−	−	+	+	+	+	−	−	绵羊、山羊	
	3	−	−	+	+	+	+	+	−	绵羊、山羊	
牛种 *	1	(+)	+	+	−	+	+	−	−	牛	
	2	(+)	+	+	−	−	+	−	−	牛	
	3	(+)	+	+	+**	+	+	−	−	牛	
	4	(+)	+	+	−	(+)	−	+	−	牛	
	5	−	−	+	+	+	−	+	−	牛	
	6	−	−	+	+**	+	+	−	−	牛	
	9	−	+	+	+	+	−	+	−	牛	
猪种	1	−	+	+	+	(−)	+	−	−	猪	
	2	−	−	+	+	−	+	−	−	猪、野兔	
	3	−	−	+	+	+	+	−	−	猪	
	4	−	−	+	+	(−)	+	+	−	驯鹿	
	5	−	−	+	+	−	−	+	−	啮齿动物	
沙林鼠种	−	−	+	+	−	−	+	−	−	沙林鼠	
犬种	−	−	−	+	+	(−)	−	−	+	犬	
绵羊附睾种	−	+	−	−	+	(−)	−	−	+	绵羊	
海洋种 ***	1	+	−	+	+	+	+	+/−	−	海豹	
	2	−	−	+	+	+	+	+/−	−	鲸	

+ 阳性；（+）通常阳性；　— 阴性；（−）通常阴性；*. 牛种 7 型、8 型不再被认可；A；牛种；M；羊种；R；粗糙型；**. 牛 3 型在 1/25000 浓度下生长，牛 6 型则不生长；***. 海洋种包括几个不同的型并且每个型都可给予族名

表 D-2　布鲁氏菌种的氧化代谢

种	氨基酸						
	丙氨酸	天门冬氨酸	谷氨酸	精氨酸	瓜氨酸	赖氨酸	鸟氨酸
羊种	+	+	+	−	−	−	−
牛种	+	+	+	−	−	−	−
猪种	V[(1)]	V[(1)]	V[(1)]	+	+	V[(1)]	+
沙林鼠种	V	+	+	−	−	−	−
犬种	V	−	+	+	+	+	+
绵羊附睾种	V	+	+	−	−	−	−
海洋种	−	−	+	−	ND	−	−

种	糖类					
	阿拉伯糖	半乳糖	核糖	木糖	葡萄糖	赤藓醇糖
羊种	−	−	−	−	+	+
牛种	+	+	+	V	+	+
猪种	V[(1)]	V[(1)]	+	+	+	+
沙林鼠种	+	+	V	−	+	+
犬种	V	V	+	−	+	V
绵羊附睾种	−	−	−	−	−	−
海洋种	ND	V[(1)]	+	+	+	+

+ 阳性；− 阴性；V 不同菌株之间不同；V[(1)] 一些有助分类的种间不同；ND 无资料

表 D-3　光滑型（S）和粗糙型 (R) 布鲁氏菌种噬菌体的噬菌活性

噬菌体群	噬菌体型	牛种		猪种		羊种	沙林鼠种		犬种	绵羊附睾种	海洋种	
		S	R	S	R	S	R	S	R	R	R	S
1	Tb	L	NL	NL	NL	NL	NL	NL/PL	NL	NL	NL	NL
2	Fi75/13	L	NL	PL	NL	NL	NL	L	NL	NL	NL	L
3	Wb	L	NL	L	NL	V	NL	L	NL	NL	NL	L
4	BK_2	L	NL	L	NL	L/PL	NL	L	NL	NL	NL	L
5	R/C	NL	L	NL	NL	NL	NL	NL	NL	L	L	NL
6	Lz_1	L	NL	bg1,4：L bg2,3，5：PL	bg1,4：V bg2,3，5：NL	L/PL	V	L	PL	NL	NL	L
7	Np	L	NL	NL	NL	NL	NL	L	NL	NL	NL	ND

L. 融合；PL. 局部，单一斑块或生长抑制；NL. 不裂解；V. 可变，一些菌株裂解；ND. 无资料；bg. 生物组

附录 E　布鲁氏菌细菌学检验

一、步骤

血液（5 ～ 10ml）或其他样本。

1．液体培养基（培养血液时，每瓶 50 ～ 100ml 培养液，包含 1% ～ 2% 枸橼酸钠）或固 - 液体混合培养基（双向培养）恒温 37℃，直至出现布鲁氏菌生长。

2．将第一代菌传代培养至琼脂培养基。

必要时，持续如上温度恒温培养 4 ～ 5d 以形成菌落。

3．用布鲁氏菌血清抗体玻片凝集试验检验菌落的“光滑 - 粗糙”程度。

4．布鲁氏菌确证试验采用传代培养的标准分型检验。

二、培养基

固体和液体培养基均可以使用。两种培养基均可以由相似成分制备。高质量的蛋白胨是必不可少的基础培养基。胰蛋白胨大豆肉汤培养基（BBL®），细菌用蛋白胨 ®（Difco Laboratories GmbH），胰蛋白酶大豆培养基（Gibco），胰蛋白胨大豆肉汤培养基（Oxoid Ltd），胰蛋白酶解酪蛋白（bioMérieux）都是合适的培养基。制备液体培养基，可将无菌马或牛血清（必须没有布鲁氏菌抗体）和葡萄糖添加到冷却至 56℃的、高压过的蛋白胨溶液中，最终浓度分别达到 5%（V/V）和 1%（W/V）。对于固体培养基，将 1.5% 浓度的琼脂加入到蛋白胨溶液后高压处理。

1．选择性培养基：改良布罗迪（Brodie）和辛顿（Sinton）液体培养基。将抗生素加入血清葡萄糖肉汤，最终达到如下浓度。

杆菌肽	25	U/ml
环己酰亚胺	100	μg/ml
多黏菌素 B	6	U/ml
萘啶酸	5	μg/ml
万古霉素	20	μg/ml
两性霉素 B	1	U/ml
D- 环丝氨酸	100	U/ml

2．选择性培养基：法雷尔培养基（Farrell）。

此固体选择性培养基制作方法是将抗生素添加到融化后冷却至 56℃的血清葡萄糖琼脂培养基中，达到下列浓度。

杆菌肽	25	U/ml
环己酰亚胺	100	μg/ml
多黏菌素 B	5	U/ml
万古霉素	20	μg/ml
萘啶酸	5	μg/ml
制霉菌素	100	U/ml

三. 双相培养基

卡斯塔涅达型的双相培养基，无论非选择性血清葡萄糖肉汤培养基还是血清葡萄糖琼脂培养基都可以用液体和固体介质制备。如果需要选择性培养基，则在改良布罗迪培养基和辛顿培养基中加入抗生素。制备如下。

将 12 ～ 14ml 融化的琼脂培养基（已添加超过 2.5% 的琼脂）覆盖在 125ml 的瓶底中，高压灭菌后，放置至变硬，将 15ml 含 1% ～ 2% 枸橼酸钠的灭菌培养液无菌条件下添加到培养瓶中。此时培养瓶一侧有固体培养基，瓶底有液体培养基。样本加入到液体培养基里，直立瓶子培养。每 24 ～ 48 小时把培养液在琼脂表面覆盖几分钟，然后将培养瓶继续直立培养。最后布鲁氏菌菌落将在培养液里和琼脂上共同生长。

四. 培养

必须考虑到培养牛种布鲁氏菌需要空气中 CO_2 浓度达到 10% ～ 20%，故开始时要一式两份进行培养，一份放在普通空气中，另一份放在添加了 CO_2 的空气中，直至观察到普通空气中菌落生长，此时不再需要向培养基提供额外的 CO_2。大部分情况下，会在 1 ～ 2 周出现阳性培养结果，但仍然建议 4 ～ 6 周后未出现阳性结果再放弃培养。

附录 F　血清学检验

一. 试管凝集试验

本试验在容量 1 ～ 2ml 清洁的玻璃或塑料试管中进行，将 0.8ml 石碳酸盐水（0.5% 石碳酸加入 0.15M 的盐水）加入到第 1 管，其余 5 ～ 10 个管加入 0.5ml，再向第 1 管加入 0.2ml 血清，混匀后，将 0.5ml 混合液移至第 2 管，接着将 0.5ml 移至第 2 管后面的管，进行一系列倍比稀释，用酚盐溶液将同等体积的标准牛种凝集悬液稀释到工作浓度，添加到除第 1 个管外的每个管，然后将试管放置 37℃温箱 20h。

根据抗原稀释工作浓度 1/4，2/4 和 3/4 制备透明度标准，相当于 25%，50% 和 75% 凝集。酚盐水用于 100% 凝集质控，未稀释抗原作为 0% 凝集质控。血清稀释凝集度得分结果（1+=25%，2+=50%，3+=75%，4+=100%）。在每组检测中，根据国际标准牛种血清（ISABS）标化阳性血清。以便在不同实验室可以比较结果。滴度解释必须依据病史和职业背景。

未免疫的牛试管凝集试验滴度 50U 及以上、免疫的牛 100U 及以上认定为感染。

用染色抗原的微量凝集反应方法可以用微滴定平板替代试管。

二. 二巯乙醇凝集试验

二巯乙醇凝集试验是将 0.1ml 血清与 0.4ml 生理盐水（0.15M）混合并加入 0.5ml 的二巯乙醇盐水（0.2M）。将血清放置 37℃温箱 1 h，然后用盐水连续倍比稀释。用盐水（无苯酚）将 0.5ml 标准试管凝集试验抗原稀释到工作浓度，再添加到每个试管中，随后进行标准检测。该试验与活动性感染密切相关，但没有标准试管凝集试验敏感。

该检测有助于区别人暴露于交叉反应抗原而产生的 IgM 凝集素和与感染有关的 IgG 凝集素。

三. 抗人球蛋白凝集试验

该血清学检验根据推荐的程序操作。37℃存放后，试管最好 4℃条件下离心，丢弃上清液，用 0.15M 氯化钠溶液再悬浮细胞沉积物，再离心。这个过程至少重复 2 次。最终用 0.5ml 抗人 IgG 血清将细胞沉积物稀释到工作浓度。试管再放置 37℃过夜。为凝集试验进行凝集评分应设置人阳性血清对照和盐水阴性对照。

如果检测 IgA 抗体，应使用特异的抗人免疫球蛋白试剂。ELISA 试验很大程度上已取代了该检测方法。

四. 虎红凝集试验（RBT）

血清样本筛查可以使用虎红凝集试验或卡片试验，即在白色瓷砖或搪瓷板上划出大约

直径 2cm 区域，将血清（0.03ml）与等量的抗原混合，室温下轻轻搅拌混合物 4min 后观察凝集。任何可见的反应都认为是阳性的。该检测很敏感，因此，阳性样本应由 CFT 试验或 ELISA 等 IgG 特异检测进行复核。尤其在急性感染的早期会出现假阴性反应。虎红凝集试验可用于所有动物，此外阳性结果应被定量试验确证。免疫动物可出现假阳性结果。假阴性结果通常出现在绵羊、山羊和猪中。

五. 补体结合试验（CFT）

此实验有诸多操作方法，但无论选择哪个操作过程，检测都必须使用光滑牛种布鲁氏菌制备的抗原，例如 S99 株或 1119-3，即抗国际标准血清的抗原。经过特殊制备的补体结合试验抗原或用于标准凝集试验的抗原，在标准化之前应用 CFT 缓冲液将原液稀释 200 倍方可使用。在补体结合试验中，浓缩抗原混悬液的细胞压积在未按照第二国际标准参考血清标化前的浓度不能超过 2%，如下所述。苯酚浓度不得超过 0.5%。当用盐水按照 1∶10 的比例稀释苯酚后，抗原混悬液呈白色，无聚合物，或在 37℃环境下 18h 后出现沉淀，此时，试验不应出现抗补体结合现象。不可将抗原冷冻储存。

微量滴定法是一种简单便捷的试验方法。CFT 的缓冲液可以按照下述方法制备，将氯化钠溶液 (42.5g)，巴比妥酸 (2.875g)，戊巴比妥钠 (1.875g)，镁硫酸盐 (1.018g)，和氯化钙 (1.147g) 在 1L 蒸馏水添加 4L0.04% 的明胶溶液。

指示系统是用同等体积的兔抗绵羊红细胞血清致敏的 3% 新鲜绵羊红细胞悬浮液。当新鲜豚鼠补体 1/30 稀释时，抗绵羊红细胞血清应是能够使细胞 100% 溶解的最小浓度的 5 倍。

后者被单独滴定以判定使绵羊红细胞悬液 100% 溶解的最小浓度；这被确定为补体单位。在第二国际标准血清的 1/200 稀释时，布鲁氏菌凝集标准检测抗原应用 CFT 缓冲液稀释成有 50% 补体固定（1.25U）。试验血清用等量 CFT 缓冲液稀释后置 58℃环境下 50min。

试验程序

1．使用 96 孔 U 底微滴定平板，第一、二排孔中放稀释的检测血清 25μl，除第一排外的所有孔中放 CFT 缓冲液 25μl。

2．将 25μl 血清从第二排向前连续倍比稀释。

3．将稀释到工作浓度的抗原 25μl 和 1.25 单位浓度的补体 25μl 添加到每个孔中，控制孔中只包含稀释液，血清 + 补体 + 稀释液，抗原 + 补体 + 稀释液，补体 + 稀释液，使每个样本共包含 75μl 液体。

4．将平板搅拌 10min 后 37℃放置 30min，或 4℃过夜。

5．将致敏红细胞（SRBC）悬浮液 25μl 添加到每个孔中，搅拌平板 10min 后 37℃放置 30min。

6．再将平板 4℃放置 2 ～ 3h，让未溶的细胞沉淀后读取结果。

溶血程度是与标准 0%,25%，50%,75% 和 100% 细胞溶解比较其一致程度。结果要用国际单位表示，即计算得出与国际标准血清平行的滴度。通常，血清固定滴度≥ 20 ICFTU/ml，被认为是阳性。然而分析人血清试验结果时，应考虑病史。

六. 间接 ELISA

目前有很多改良的间接 ELISA 可用于商业检测。只有使用光滑型牛种或羊种布鲁氏菌脂多糖的 ELISA 是被推荐的。检测操作用 96 孔平底聚苯乙烯微平板。由于是观察背景结果，故微平板的选择对检测性能有轻微影响。中低蛋白结合微孔板对使用 LPS 抗原也有影响。

抗原包被液即 0.05M 碳酸 - 磷酸氢盐溶液，pH 9.6，由 $NaHCO_3$ (2.93g)，Na_2CO_3 (1.59g) 和 NaN_3(0.2g) 加入到 1L 蒸馏水或去离子水中构成。结合和检测血清稀释缓冲液是 0.01 M PBS,pH 7.2, + 0.05%(V/V) 吐温 20，Na_2HPO_4 (1.21g)，KH_2PO_4 (0.20g)，NaCl (8.00g)，和 KCl (0.20g) 加入 1L 蒸馏水或去离子水 + 0.50ml/L 的吐温 20。洗液是 0.002 M PBS，pH7.4 + 0.05% 吐温 20。

结合使用应该将对人 IgG 重链和轻链特异的多克隆抗体与辣根过氧化物酶结合。基质系统是将 4.4mM H_2O_2 和 3.6mM 2,2- 联氮 - 二 (3- 乙基 - 苯并噻唑 -6- 磺酸) 二铵盐 (ABTS) 加入 0.05M 磷酸盐 / 柠檬酸盐缓冲液中，pH4.5，由 0.2M Na_2HPO_4 (25.7ml)，0.1M 柠檬酸 (24.3ml) 和蒸馏水或去离子水 (50ml) 组成；如有必要调整 pH。酶促反应停止液是 4% 十二烷基硫酸盐或将 0.1 M NaN_3 加入蒸馏水或去离子水中。

试验程序

1．将脂多糖抗原用缓冲液稀释，其浓度视棋盘式滴定而定，通常为 1μg/ml，分配到所有微孔 100μl，微板放 37℃ 2h 或 4℃过夜。由于这是固相 ELISA 技术，从每个试验步骤到移除未绑定或未反应试剂时微板孔需要洗涤。需要 3 ～ 4 次的洗脱循环，添加下一个试剂前，平板应该调转并用无毛的吸收剂拍打表面清除其他剩余物。

2．血清学检验和质控被用稀释液稀释成 1/200，置于每孔 100μl，将平板盖上并密封，放置轨道板振动器 37℃连续震动 1h，如上方法清洗平板。

3．用稀释液稀释酶标记物，置于每个孔中 100μl，将平板盖上并密封，放轨道板振动器 37℃连续振动 1h。标记物的最佳稀释应该是当标准条件下，与质控有强阳性反应时，导致 1 ～ 1.4 吸收单位的平均吸光度（见步骤 6）。应使用已知参考血清。如上清洗平板。

4．新鲜的基质 / 色原溶液制备是将 60μl 的 3%H_2O_2 原液加入 12ml 包含 3.6mM ABTS 的磷酸 / 柠檬酸缓冲液中。将基质 / 色原溶液分配到所有微孔 100μl。将平板放置轨道板振动器 37℃连续振动 15min。之后将所有微孔加入停止液 100μl，再将平板放置轨道板振动器短暂振动，确保充分混合。此时所有孔中含 200μl 液体。

5．使用 405nm 或 414nm 干涉滤光片的微平板光度计读取显色。

6．数据可以用许多不同的方式表达，但建议血清学反应用标化的强阳性血清质控的阳性百分比表达。强阳性质控血清应该如此：用阴性血清预稀释时，展现出的抗体活性位于原始高滴度血清的剂量反应曲线的线性部分，略低于平台期。

附录 G　防控布鲁氏菌病的跨部门协作策略

一. 策略

任何控制项目的成功实施都需要社区的多部门合作。开展防控项目需要社区具备有效、实际、经济的控制基础。应根据社区规划的需求来制定国家综合项目实施细则。项目开展策略概况如下。

- 预防畜间感染播散，监测无布鲁氏菌病畜群及地区。
- 在指定畜群和地区开展大规模免疫接种以降低感染率。
- 控制感染播散，除特定措施外还实施非特定措施，或在特定措施不可行的地区实施非特定措施。
- 通过检疫和屠宰来彻底清除感染动物，以扩大清洁牛 / 羊群和地区。
- 通知和教育公众，培训专业人员（表 G-1）。

这些策略并不互相排斥，多数有效的项目能把这些元素结合起来。

二. 实用方法

可以应用多种适合现场条件的技术来实施项目，包括：

- 疫情分析（详见第 8 章，表 4）。
- 牛群 / 羊群和行动地区的选择。
- 低流行区和清洁区的保护（详见世界动物卫生组织国际动物卫生法规，1997）。

三. 防控措施的计划、管理与实施

（一）社区防控措施的开始与后续步骤

1．指定负责人。

2．资源分配。

3．每年评估社区计划。

4．与当地政府共同确认下一步具体措施，包括讨论关于流行病学评估和防控议程的行动方案等。

5．要求不同国家部门间合作。

（二）实施社区—政府援助启动计划

1．制定详细决策指南。

2．明确当地行动计划，包括：

（1）流行病学调查。

（2）个人卫生的教育和信息传播。

（3）免疫规划。

（4）动物更替方案。

（三）多种活动和服务的发展

1．诊断服务。

2．使用冷链的疫苗供给。

3．动物废物处理或补偿。

4．报告中央政府项目进展。

（四）国家综合规划

1．建立跨部门的委员会。

2．指定国家项目指挥部。

3．编写指南，用于：

（1）社区活动。

（2）支持服务。

（3）国家综合规划。

4．回顾和改进国家规章条例。

5．规划全国布鲁氏菌病控制项目。

6．组织机构。

7．资源调度。

8．规划实施的不同阶段，包括下列方面：

（1）财政资源。

（2）地理覆盖。

（3）技术。

（4）人力资源。

9．项目监测、定期评估和回顾。

若要取得成功，社区里所有团队中的每个人都必须了解项目状态。

（五）国际合作

在布鲁氏菌病防控技术方面，世界卫生组织、联合国粮农组织、世界动物卫生组织都鼓励和支持与上述国际布鲁氏菌病控制策略相结合的项目。关于人布鲁氏菌病的建议可以来自世界卫生组织。关于农业和国际贸易方面的建议来自联合国粮农组织和世界动物卫生组织。

表G-1　布鲁氏菌病健康教育主题

分组	主题	预期行动
牲畜饲养者	1. 布鲁氏菌病的概念 2. 疾病特征 3. 对人健康的损害 4. 对动物产品的危害 5. 法规支持采取的措施	由卫生和畜牧部门提供防控布鲁氏菌病的合作措施
与动物直接接触的专业人员（放牧者、农民、牛奶工、屠宰工、配种员、兽医）	1. 布鲁氏菌病的概念 2. 疾病特征 3. 对人健康的损害 4. 影响物种 5. 传播给人的途径 6. 预防措施，例如使用防护衣、个人卫生、环境卫生	应用预防措施
普通人群	1. 布鲁氏菌病的概念及其危害 2. 传播给人的途径 3. 临床症状 4. 预防方法，尤其是与奶或鲜奶酪食用相关的方法	对关心自身健康和知晓自己可能患布鲁氏菌病抱有积极态度

来源：世界卫生组织 / 联合国粮农组织布鲁氏菌病专家联合委员会．第六报道，技术报告系列，No 740，世界卫生组织，日内瓦，1986.

附录 H　预防和控制人间布鲁氏菌病推荐监测标准（A23）

一、概述

布鲁氏菌病是一种广泛传播的人兽共患病，主要传染源是牛、绵羊、山羊、猪、骆驼，通过直接接触血液、胎盘、胎儿或子宫分泌物，或食用生的受污染的动物制品（尤其是未经消毒的牛奶和软奶酪）传播。在流行地区，人布鲁氏菌病存在严重的公共卫生影响。世界范围内，免疫接种散养的绵羊和山羊存在困难，在一些国家通过免疫和（或）淘汰病畜消除畜间布鲁氏菌病不可行，此时预防人间布鲁氏菌病主要基于增强意识、食品安全措施、职业卫生和实验室安全。大部分国家布鲁氏菌病是法定传染病。

二、传染源和主要传播途径

1．传染源　牛种 1 ～ 6,9 型；羊种 1 ～ 3 型；猪种 1 ～ 4 型；犬种；较少有海洋种感染人的报道。被感染动物（主要是牛、绵羊、山羊、猪、犬等）及其制品是宿主和传染源。

2．主要传播途径　通过食用、破损皮肤直接接触和空气传播（实验室和屠宰场）感染。主要感染食用生奶及其制品的消费者、农民、屠宰工、兽医和实验室人员。潜伏期 1 周到 2 个月，甚至更长，通常 2 ～ 4 周。

三、临床描述和推荐的病例定义

临床描述。布鲁氏菌病可以急性或慢性发作，持续、间歇或不规则发热，多汗，乏力，食欲不振，消瘦，头痛，关节痛和全身酸痛。形成脓肿是罕见的并发症。多数死亡由于布鲁氏菌性心内膜炎和神经性布鲁氏菌病。

四、实验室标准

（一）疑似诊断

1．虎红凝集试验（RBT）用于筛查；筛查阳性后通过下列确证试验之一进行确诊。

2．试管凝集试验（SAT）。

（二）确证诊断

1．从血清或其他临床样本中分离布鲁氏菌。

2．基于凝集抗体检测（RBT，SAT）的疑似实验室诊断结合非凝集抗体检测，通过：

— ELISA IgG 的检测。

— 抗人球蛋白试验 IgG。

PCR 和侧向层析检测等新的快速检测方法尚未被认可。

五、病例分类（人）

1．*疑似病例*　与临床描述一致并且流行病学与疑似 / 确诊患病动物或污染的动物制品相关。

2．*可能病例*　疑似病例合并疑似实验室诊断。

3．*确诊病例*　疑似病例或可能病例合并确诊实验室诊断。

六、监测

1．*监测原理*　监测是预防控制项目管理的关键要素。

2．*推荐的监测类型*　医护人员或实验室人员将病例尽快报告给上级公共卫生和相关畜牧部门。在疫情流行国家对所有报告病例展开调查可能不现实，因此应对典型病例展开调查。

七、推荐的最小数据元素

（一）病例基本数据

1．*病例分类*。

2．编码、年龄、性别、地理信息和职业。

（二）*汇总数据报告*

分类的病例数（可能病例 / 确诊病例）、年龄、性别、地理信息和职业。

八、推荐的数据分析、描述和报告

1．*图*　可能病例数 / 确诊病例数的月分布。

2．*表*　可能病例数 / 确诊病例数的年龄、性别、月、地点分布。

3．*地图*　可能病例数 / 确诊病例数的地区分布。

九、监测指标

1．报告的及时性和完整性。

2．疑似、可能、确诊病例的比例。

3．流行病学调查数与病例数之比。

十、控制活动

（一）病例管理

多西环素每天 2 次，每次 100mg，治疗 45d 加链霉素每天 1g，治疗 15 天。主要替代疗法是多西环素每天 2 次，每次 100mg，治疗 45d 加利福平 [15mg/（kg • d）(600 ～ 900mg)] 治疗 45 天。经验建议是链霉素可以被庆大霉素替代 [5mg/（kg • d），应用 7 ～ 10 天]，但没有研究直接比较两种方案的一般疗效。孕妇、婴儿和未满 8 岁的儿童的最佳治疗方案尚未确定。治疗儿童的经验是复方磺胺甲噁唑结合氨基糖苷类药物（链霉素、庆大霉素）或利福平。

（二）预防

1．建议避免食用未经消毒的奶及奶制品。

2．猎人和有风险的专业人员（肉贩、农民、屠夫、兽医）采取防护措施。

3．仔细处置胞衣（胎盘和羊膜），尤其在发生流产时。

4．动物血清学和其他检验；牛群或羊群的免疫；感染牛群或羊群的淘汰。

十一、疫情流行

1．污染产品的分销

这些产品通常来自感染牛，羊群的生奶和奶酪。可能出现流行的情况。

2．流行的管理

确认共同的传播媒介，召回污染产品，引进巴氏灭菌前停止生产和销售产品。

十二、耐药性监测

不适用。

十三、控制活动的考核指标

每 10 万人新发病例数。

十四、其他方面

特殊要求 / 其他干预措施。预防控制畜间布鲁氏菌病的最成功方法是免疫。在卫生和畜牧部门间相互协调与合作，即确保各部门间联合决策以促进信息即时共享，以及联合调查、控制、公众健康教育。

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Brucellosis in humans and animals

Food and Agriculture Organization of the United Nations

World Organisation for Animal Health

World Health Organization

Contents

Principal author

Dr MJ Corbel, Division of Bacteriology, National Institute for Biological Standards and Control, Potters Bar, Hertfordshire, EN6 3QG, England.

List of contributors

The late Dr GG Alton, New Friars, Mickley, Ripon, Yorkshire HG4 3JE, UK.

Dr J Ariza, Infectious Diseases Service, Hospital de Bellvitage, University of Barcelona, Spain.

Dr M Banai, Kimron National and OIE Reference Laboratory for Brucellosis, Kimron Veterinary Institute, PO Box 12, Bet Dagan 50250, Israel 50250.

Dr O Cosivi, Department of Epidemic and Pandemic Alert and Response, World Health Organization, 1211 Geneva 27, Switzerland.

Dr R Diaz, Department of Microbiology, University of Navarra, Apatdo 273, 31081 Pamplona, Spain.

Professor EA Dranovskaya, formerly at N F Gamalei Institute of Epidemiology and Microbiology, u1. Gamalei, 18, 123098 Moscow D98, Russia Federation.

Professor SS Elberg, 2853 Oak Court, Ukiah, CA95482, USA.

Dr B Garin-Bastuji, National & OIE/FAO Animal Brucellosis Reference

Laboratory, French Agency for Food Safety, 23, av. du Général-de-Gaulle, 94706 Maisons-Alfort Cedex, France.

Dr J Kolar, Pod Belohorska 30, 15000 Prague 5, Czech Republic.

Dr AP MacMillan, WHO/FAO Collaborating Centre for Reference and Research on Brucellosis, Veterinary Laboratory Agency, New Haw, Addlestone, Surrey KT15 3NB, UK.

Professor A Mantovani, Isituto Superiore di Sanità, WHO/FAO Collaborating Centre in Veterinary Public Health, viale Regina Elena 299, 00161 Rome, Italy.

Dr I Moriyon, Department of Microbiology, University of Navarra, Aptdo 273 31081 Pamplona, Spain.

Dr A Mousa, A1-Adam Hospital, Ministry of Public Health, P.O. Box 46940, 64020 Fahaheel, Kuwait.

Dr P Nicoletti, Institute of Food & Agricultural Sciences, College of Veterinary Medicine, Department of Infectious Diseases, University of Florida, Building 471, Mowry Road, P O Box 110880, Gainesville FL 32611 0880, USA.

Dr A Semeinis, WHO Mediterranean Zoonoses Control Centre, 24, Stournari str. 10682, Athens, Greece.

Dr EJ Young, Department of Veterans Affairs, Medical Center, Holcombe Boulevard, Houston, Texas, USA.

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Abbreviations

2-ME	2-mercaptoethanol
BCV	*Brucella* chemical vaccine
CF	complement fixation
CFT	complement fixation test
CIEP	counter-immunoelectrophoresis
CNS	central nervous system
CSF	cerebrospinal fluid
CT	computed tomography
DNA	deoxyribonucleic acid
DTT	dithiothreitol
ELISA	indirect enzyme-linked immusorbent assay
IU	international unit
LPS	lipopolysaccharide
MRT	milk ring test
NMR	nuclear magnetic resonance
PCR	polymerase chain reaction
RBT	Rose Bengal plate test
RES	reticuloedothelial system
SAT	serum agglutination test
S-LPS	smooth lipopolysaccharide
SMZ	sulfamethoxazole
TMP	trimethoprim

1. Introduction

Brucellosis, also known as “undulant fever”, “Mediterranean fever” or “Malta fever” is a zoonosis and the infection is almost invariably transmitted by direct or indirect contact with infected animals or their products. It affects people of all age groups and of both sexes. Although there has been great progress in controlling the disease in many countries, there still remain regions where the infection persists in domestic animals and, consequently, transmission to the human population frequently occurs. It is an important human disease in many parts of the world especially in the Mediterranean countries of Europe, north and east Africa, the Middle East, south and central Asia and Central and South America and yet it is often unrecognized and frequently goes unreported.[1] There are only a few countries in the world that are officially free of the disease although cases still occur in people returning from endemic countries.

It is a zoonosis and the infection is almost invariably transmitted to people by direct or indirect contact with infected animals or their products. Although there has been great progress in controlling the disease in many countries, there still remain regions where the infection persists in domestic animals and, consequently, transmission to the human population frequently occurs.

Expansion of animal industries and urbanization, and the lack of hygienic measures in animal husbandry and in food handling partly account for brucellosis remaining a public health hazard. Expansion of international travel which stimulates the taste for exotic dairy goods such as fresh cheeses which may be contaminated, and the importation of such foods into *Brucella*-free regions, also contribute to the ever-increasing concern over human brucellosis.

The duration of the human illness and its long convalescence means that brucellosis is an important economic as well as a medical problem for the patient because of time lost from normal activities. Prompt diagnosis and treatment with antibiotics has greatly reduced the time a patient may be incapacitated. Nevertheless, there are many regions where effective diagnosis or treatment is not available and/or where programmes for the detection and prevention of the infection in humans and animals are not adequately carried out. In these areas, the animal disease remains a constant threat to human welfare, particularly for those in the most vulnerable socioeconomic sections of the population.

The disease can be insidious and may present in many atypical forms. In many patients the symptoms are mild and, therefore, the diagnosis may not be even considered. Indeed it should be noted that even in severe infections differential diagnosis can still be difficult. The application of well-controlled laboratory procedures and their careful interpretation can assist greatly in this process.

While there is still a need for technical advances in some areas, it is important to note that the basic scientific information and methods required for the control of brucellosis in ruminants are at hand. Even where brucellosis in animals is not under control there are measures that can be taken to prevent human infection and to treat infected persons.

Intersectoral cooperation in support of primary health care approaches plays an important role in the control of brucellosis and may contribute to the development of appropriate infrastructures in areas of animal production, food hygiene, and health care. On the other hand the prevention and control of brucellosis needs supportive action from various sectors, including those responsible for food safety and consumer education.

1 World animal health data, including brucellosis in animals and humans, are contained in Handistatus II and are also available in a hardcopy publication entitled World Animal Health. This information is collected from Veterinary Services of OIE, FAO and WHO Member Countries by the OIE Central Bureau, Paris, France, using a joint annual questionnaire and can be accessed though the following address: http://www.oie.int

Emphasis in this document is placed on fundamental measures of environmental and occupational hygiene in the community and in the household as well as on the sequence of actions required to detect and treat patients.

2. Clinical manifestation

Brucellosis is essentially a disease of animals, especially domesticated livestock, caused by bacteria of the *Brucella* group with humans as an accidental host. In other words it is a zoonosis. On genetic grounds the *Brucella* group can be regarded as variants of a single species which for historical reasons is identified as *Brucella melitensis.* However, for practical purposes this approach is considered unsatisfactory and six main "species" are distinguished: *B. abortus, B. suis, B. melitensis, B. neotomae, B. ovis, B. canis.* Strains isolated from marine mammals fall into at least three groups distinct from these and may be designated as new "nomen species".

The differentiation of these variants is of practical importance as the epidemiology and, to a lesser extent, the severity of the disease in humans, is influenced by the type of organism and its source. Thus *B. abortus* is normally associated with cattle, *B. melitensis* with sheep and goats, *B. suis* with swine (although biovars 4 and 5 are specifically associated with reindeer and rodents respectively). *B. ovis* causes an infection specific for sheep and has not been conclusively implicated in human disease, *B. suis* biovar 5 has only been isolated on a few occasions from rodents and *B. canis is* usually associated with disease in dogs but occasionally causes human brucellosis. *B. neotomae* has been isolated on few occasions and has never been implicated in human disease.

The human disease usually manifests itself as an acute febrile illness which may persist and progress to a chronically incapacitating disease with severe complications. It is nearly always acquired directly or indirectly from animal sources, of which cattle, sheep, goats and pigs are by far the most important. In these natural hosts, the infection usually establishes itself in the reproductive tract, often resulting in abortion. Excretion in genital discharges and milk is common and is a major source of human infection.

The clinical picture is not specific in animals or humans and diagnosis needs to be supported by laboratory tests. Effective treatment is available for the human disease but prevention is the ideal, through control of the infection in animals and by implementation of hygienic measures at the individual and public health levels.

2.1 The disease in humans

Brucellosis is an acute or sub-acute febrile illness usually marked by an intermittent or remittent fever accompanied by malaise, anorexia and prostration, and which, in the absence of specific treatment, may persist for weeks or months. Typically, few objective signs are apparent but enlargement of the liver, spleen and/or lymph nodes may occur, as may signs referable to almost any other organ system. The acute phase may progress to a chronic one with relapse, development of persistent localized infection or a non-specific syndrome resembling the "chronic fatigue syndrome". The disease is always caused by infection with a *Brucella* strain and diagnosis must be supported by laboratory tests which indicate the presence of the organism or a specific immune response to its antigens.

Evidence in support of the diagnosis includes:

- A history of recent exposure to a known or probable source of *Brucella* spp. This includes common host species, especially cattle, sheep, goats, pigs, camels, yaks, buffaloes or dogs; consumption of raw or inadequately cooked milk or milk products, and, to a lesser extent, meat and offal derived from these animals. In addition, the resistance of the organism and its high infectivity make environmental contamination a probable hazard, although this is always difficult to prove. Occupational exposure and/or residence in an area

in which the infection is prevalent, also raise the probability of the diagnosis.

- Isolation of *Brucella* spp. from the patient.
- Demonstration by validated polymerase chain reaction (PCR) of the presence of *Brucella* genetic material in blood or other tissue sample.
- Demonstration by a validated serological method of *Brucella* antigen in blood or other tissue sample.
- Demonstration of a rising antibody titre in any serological test for brucellosis in the absence of exposure to any known source of cross-reacting antigens.
- Demonstration of a high sustained IgG antibody titre in the agglutination, complement fixation or ELISA tests with standardized antigens.

Susceptibility to brucellosis in humans depends on various factors, including the immune status, routes of infection, size of the inoculum and, to some extent, the species of *Brucella*. In general, *B. melitensis* and *B. suis* are more virulent for humans than *B. abortus* and *B. canis*, although serious complications can occur with any species of *Brucella*.

Common routes of infection include direct inoculation through cuts and abrasions in the skin, inoculation via the conjunctival sac of the eyes, inhalation of infectious aerosols, and ingestion of infectious unpasteurized milk or other dairy products. Blood transfusion, tissue transplantation and sexual transmission are possible but rare routes of infection.

The disease is acute in about half the cases, with an incubation period of two to three weeks. In the other half, the onset is insidious, with signs and symptoms developing over a period of weeks to months from the infection. The clinical manifestations are varied and nonspecific. They include fever, sweats, fatigue, malaise, anorexia, weight loss, headache, arthralgia and back pain. Commonly, patients feel better in the morning, with symptoms worsening as the day progresses. The desire to rest can be profound, and depression is pervasive. If untreated, the pattern of the fever waxes and wanes over several days (“undulant fever”). Table 1 reports symptoms and signs in 500 patients with brucellosis due to *B. melitensis*.

Table 1 Symptoms and signs in 500 patients with brucellosis due to *B. melitensis*.

Symptoms and signs	Number of patients	%
Fever	464	93
Chills	410	82
Sweats	437	87
Aches	457	91
Lack of energy	473	95
Joint and back pain	431	86
Arthritis	202	40
Spinal tenderness	241	48
Headache	403	81
Loss of appetite	388	78
Weight loss	326	65
Constipation	234	47
Abdominal pain	225	45
Diarrhoea	34	7
Cough	122	24
Testicular pain/epididymo-orchitis	62	21[a]
Rash	72	14
Sleep disturbance	185	37
Ill appearance	127	25
Pallor	110	22
Lymphadenopathy	160	32

Symptoms and signs	Number of patients	%
Splenomegaly	125	25
Hepatomegaly	97	19
Jaundice	6	1
Central nervous system abnormalities	20	4
Cardiac murmur	17	3
Pneumonia	7	1

Adapted from MM Madkour. *Brucellosis Overview.* In: Madkour's Brucellosis, 2nd edition. Springer, Berlin

[a] Among 290 males

Brucella species are facultative intracellular pathogens that can survive and multiply within phagocytic cells of the host. The mechanisms by which *Brucella* evades intracellular killing are incompletely understood. Nevertheless, *Brucella* organisms ultimately become sequestered within monocytes and macrophages of the reticuloendothelial system (RES), such as lymph nodes, liver, spleen and bone marrow. Brucellosis is a systemic infection that can involve any organ or tissue of the body. When clinical symptoms related to a specific organ predominate, the disease is termed "localized". Commonly, localization involves organs of the RES.

Although humoral antibodies appear to play some role in resistance to infection, the principal mechanism of recovery from brucellosis is cell-mediated. Cellular immunity involves the development of specific cytotoxic T lymphocytes and activation of macrophages, enhancing their bactericidal activity, through the release of cytokines (e.g. gamma interferon and tumour necrosis factor) from specifically committed helper T lymphocytes. Coincident with the development of cell-mediated immunity, the host usually demonstrates dermal delayedtype hypersensitivity to antigens of *Brucella*.

2.1.1 Osteoarticular complications

Bone and joint involvement are the most frequent complications of brucellosis, occurring in up to 40% of cases. A variety of syndromes have been reported, including sacroiliitis, spondylitis, peripheral arthritis, osteomyelitis, bursitis, and tenosynovitis. *Brucella* sacroiliitis is especially common. Patients present with fever and back pain, often radiating down the legs (sciatica). Children may refuse to walk and bear weight on an extremity. Early in the disease, radiographs and bone scintigrams can appear normal, but, in time, computed tomography (CT) or nuclear magnetic resonance (NMR) scans may show narrowing of the intervertebral disc space. Vertebral osteomyelitis is readily apparent through radionucleide scans showing destruction of the vertebral bodies. The lumbar vertebrae are involved more often than the thoracic and cervical spine. Paravertebral abscesses are less common in brucellosis than in spinal tuberculosis. A post-infectious spondyloarthropathy involving multiple joints has been described, and is believed to be caused by circulating immune complexes.

2.1.2 Gastrointestinal complications

Brucellosis, especially when due to *B. melitensis*, is often foodborne, and unpasteurized milk or dairy products, such as cheese, are common vehicles of transmission. Foodborne brucellosis resembles typhoid fever, in that systemic symptoms predominate over gastrointestinal complaints. Nevertheless, some patients with the disease experience nausea, vomiting, and abdominal discomfort. Rare cases of ileitis, colitis and spontaneous bacterial peritonitis have been reported.

2.1.3 Hepatobiliary complications

The liver is commonly involved in brucellosis, although liver function tests can be normal or only mildly elevated. The histological changes in the liver are variable, but disease caused by *B. abortus* may show epithelioid granulomas that are indistinguishable from sarcoidosis lesions. A spectrum of hepatic lesions has been described in cases due to *B. melitensis*, including scattered small foci of inflammation

resembling viral hepatitis. Occasionally larger aggregates of inflammatory cells are found within the liver parenchyma with areas of hepatocellular necrosis. In other cases, small, loosely formed epithelioid granulomas with giant cells can be found.

Despite the extent of hepatic involvement, post-necrotic cirrhosis is extremely rare. Hepatic abscesses and chronic suppurative lesions of the liver and other organs have been described in cases due to *B. suis*. Acute and chronic cholecystitis have been reported in association with brucellosis.

2.1.4 Respiratory tract complications

Aerosol inhalation is a recognized route of transmission of brucellosis, especially common in abattoirs where infected animals are slaughtered. A variety of pulmonary complications have been reported, including hilar and paratracheal lymphadenopathy, interstitial pneumonitis, bronchopneumonia, lung nodules, pleural effusions, and empyema. *Brucella* organisms are rarely isolated from expectorated sputum.

2.1.5 Genitourinary complications

Orchitis and epididymitis are the most frequent genitourinary complications of brucellosis in men. Usually unilateral, *Brucella* orchitis can mimic testicular cancer or tuberculosis. Although *Brucella* organisms have been recovered from banked human spermatozoa, there have been a few reports implicating sexual transmission. Renal involvement in brucellosis is rare, but it too can resemble renal tuberculosis. In women, rare cases of pelvic abscesses and salpingitis have been reported.

2.1.6 Pregnancy and breastfeeding

Brucellosis during the course of pregnancy carries the risk of spontaneous abortion or intrauterine transmission to the infant. Abortion is a frequent complication of brucellosis in animals, where placental localization is believed to be associated with erythritol, a growth stimulant for *B. abortus*. Although erythritol is not present in human placental tissue, *Brucella* bacteremia can result in abortion, especially during the early trimesters. Whether the rate of abortions from brucellosis exceeds rates associated with bacteremia from other bacterial causes is unclear. In any event, prompt diagnosis and treatment of brucellosis during pregnancy can be lifesaving for the fetus.

Very rare human-to-human transmission from lactating mothers to their breastfed infants has been reported.

2.1.7 Cardiovascular complications

Infective endocarditis is the most common cardiovascular manifestation, and it is said to be the most common cause of death from brucellosis. Endocarditis is reported in about 2% of cases, and can involve both native and prosthetic heart valves. The aortic valve is involved more often than the mitral valve. Aneurysms of the sinus of Valsalva and other vascular structures appear to be most common when infection is caused by *B. suis*. Mycotic aneurysms, usually involving the middle cerebral artery, can be a neurological complication of infective endocarditis. Treatment of endocarditis caused by *Brucella* species usually requires a combination of antimicrobial therapy and valve replacement surgery.

2.1.8 Neurological complications

Neurobrucellosis refers to a variety of neurological complications associated with brucellosis. Direct invasion of the central nervous system occurs in about 5% of cases of *B. melitensis* infection, and meningitis or meningoencephalitis are the most common manifestations. *Brucella* meningitis can be acute or chronic. It often occurs late in the course of disease, but it can be the presenting manifestation. Analysis of cerebrospinal fluid (CSF) usually reveals an elevated protein content, normal or low glucose concentration,

and a lymphocytic pleocytosis. *Brucella* organisms are rarely isolated from CSF, but specific antibodies can be demonstrated in the CSF and serum. Other CNS manifestations of brucellosis include cerebral vasculitis, mycotic aneurysms, brain and epidural abscesses, infarcts, haemorrhage, and cerebellar ataxia. Peripheral nerve complications include neuropathy/radiculopathy, Guillain-Barré syndrome, and a poliomyelitis-like syndrome.

Brain scans (e.g. CT, magnetic resonance imaging) are usually normal in meningitis, but can be useful for detecting space-occupying lesions and the integrity of the epidural space. Basal ganglia calcification has been reported in some patients with neuro-brucellosis.

2.1.9 Cutaneous complications

A variety of skin lesions have been reported in patients with brucellosis, including rashes, nodules, papules, erythema nodosum, petechiae, and purpura.Cutaneous ulcers, abscesses, and suppurative lymphangitis appear to be more common with *B. suis*. Occasionally, epistaxis, gingivorrhea, haematuria, and cutaneous purpura occur in association with severe thrombocytopenia, which has been ascribed to hypersplenism, bone marrow haemaphagocytosis, and/or anti-platelet antibodies.

2.1.10 Opthalmic complications

Although uncommon, a variety of ocular lesions have been reported in patients with brucellosis. Uveitis is the most frequent manifestation, and can present as chronic iridocyclitis, nummular keratitis, multifocal choroiditis or optic neuritis. Since *Brucella* organisms have not been isolated from the structures of the eye in humans, many of these lesions are considered to be late complications, possibly immunologically mediated. Consequently, the usual treatment for ocular complications is steroids.

2.1.11 Chronic brucellosis

Perhaps no aspect of the disease elicits more controversy than chronic brucellosis. This is due, in part, to the lack of a universally accepted definition. Most authorities agree that the term “chronic brucellosis” should be reserved for patients whose clinical symptoms persist for 12 months or more from the time of the diagnosis. Using this criterion, patients fall into three categories: (1) relapse, (2) chronic localized infection, and (3) delayed convalescence.

Relapse is defined as the recurrence of characteristic signs and symptoms (with or without a positive culture) occurring at some time after the completion of a course of treatment. Patients with relapse characteristically have objective signs of infection, such as fever, and persistently elevated titres of IgG antibodies in their serum. Most relapses occur within six months after therapy is discontinued, and relapse is not usually due to the emergence of antibiotic resistant strains, although this has been seen after monotherapy with rifampicin or streptomycin. Therefore, relapse can usually be treated by repeating the course of therapy with the same drugs.

Chronic localized infection is defined as the recurrence of characteristic signs and symptoms (with or without a positive blood culture) caused by the failure to eliminate a deep focus of infection, such as osteomyelitis, or deep tissue abscesses. Patients with localized infection have also objective signs of infection, such as fever, although symptoms may recur intermittently over long periods of time. As is the case with patients with relapse, localized infection is characterized by persistent elevation of IgG antibodies in the serum. Unlike relapse, chronic localized brucellosis may require surgical intervention to drain foci of infection in addition to antimicrobial therapy.

Delayed convalescence is defined as the persistence of symptoms, without objective signs of infection, such as fever, in patients who have completed a course of therapy, and in whom titres of antibodies have declined

or even disappeared. The etiology of delayed convalescence is unknown, but psychological studies of some patients suggest a high incidence of personality disorders, often predating the onset of brucellosis. In any case, patients with delayed convalescence do not appear to benefit from repeated courses of antimicrobial therapy.

2.1.12 Childhood brucellosis

Once considered rare in children, it is now recognized that brucellosis can affect persons of all ages, especially in areas where *B. melitensis* is the predominant species. The course of infection and the incidence of complications appear to be similar regardless of the age of the patients.

KEY POINTS ON THE DISEASE IN HUMANS

- Human brucellosis usually presents as an acute febrile illness.
- Most cases are caused by *B. melitensis*.
- All age groups are affected.
- Complications may affect any organ system.
- The disease may persist as relapse, chronic localized infection or delayed convalescence.

2.2 The disease in animals

Brucellosis is a sub-acute or chronic disease which may affect many species of animals. In cattle, sheep, goats, other ruminants and pigs the initial phase following infection is often not apparent. In sexually mature animals the infection localizes in the reproductive system and typically produces placentitis followed by abortion in the pregnant female, usually during the last third of pregnancy, and epididymitis and orchitis in the male (Fig. 1 and 2). Clinical signs are not pathognomonic and diagnosis is dependent upon demonstration of the presence of *Brucella* spp. either by isolation of the bacteria or detection of their antigens or genetic material, or by demonstration of specific antibody or cell-mediated immune responses.

Brucellosis is a disease of many animal species but especially of those that produce food: sheep (especially milk-producing), goats, cattle and pigs and, on a more localized scale, camels, buffaloes, yaks and reindeer. Five of the six currently recognized *Brucella* species cause infection and clinical signs in one or more animal hosts (see Table 2). Four of these also cause human disease: *B. melitensis, B. suis, B. abortus* and *B. canis* in descending order of pathogenicity. The recently recognized types associated with marine animals may also have the capacity to cause human disease.

The *Brucellae* are somewhat host-specific but cross-species infections occur, especially with *B. melitensis*. Infections in many wildlife species have been reported but those that obviously affect population fecundity and result in human infections are quite rare. *B. melitensis* infections in dairy herds, however, have severe economic and public health implications.

Infections in sheep and goats are highly contagious because of the pathogenicity of *B. melitensis* and because of close contact caused by the density of the flocks or herds, the commingling of those of different owners and heavy exposure in housing. Animal-to-animal transmission occurs as a result of the large number of organisms shed in the environment.

Humans are often infected due to direct animal contact or ingestion of contaminated dairy products. Human

cases may be a useful indicator of the presence of disease in animal populations and may be the only source of information for surveillance. It is important, however, to determine if the infection was acquired locally or elsewhere, and, if food products are implicated, to establish whether these were locally produced or imported (see Section 8).

Characteristic but not specific signs of brucellosis in most animal hosts are abortion or premature births and retained placenta. In some areas, abortion is relatively uncommon. In some parts of Africa, hygromas and abscesses are the major clinical signs in nomadic or semi-nomadic cattle herds infected with *B. arbortus* biovar 3. There is lowered milk production due to premature births. Interference with fertility is usually temporary and most infected animals will abort only once and some are unaffected. The udder is often permanently infected, especially in the case of cows and goats. Shedding of organisms in milk is frequent. Localized infections in sheep result in orchitis or epididymitis in the case of *B. melitensis* and *B. ovis*. In goats, cattle, swine and dogs similar complications may follow infection with *B. melitensis, B. abortus, B. suis* and *B. canis* respectively. Arthritis may also be a rare sign in *B. melitensis*-infected sheep and goats. In horses, local abscess formation in bursae may be the only clinical sign and infection in this species is often asymptomatic. Camels infected with *B. melitensis* shed the organisms in milk and in some countries this is a serious public health problem. Clinical signs of brucellosis in camels appear to be very rare.

The severity of the disease depends upon many factors such as previous vaccination, age, sex and management such as herd or flock size and density. Abortions are more prevalent in unvaccinated animals and numbers of organisms shed are much greater. The bacteria are found in tissues and fluids associated with pregnancy, the udder and the lymph nodes which drain the relevant areas.

Most infections result from ingestion of bacteria either from diseased animals or contaminated feedstuffs. However, infection may also be acquired by respiratory exposure and by contamination of abraded skin and mucosal surfaces. Natural breeding transmits infection in swine and dogs and, to a lesser extent, sheep and goats. Persistent bacteraemias are also more common in the first two species. Bacteraemia occurs during the course of infection in other species but is usually intermittent and of short duration.

KEY POINTS ON THE DISEASE IN ANIMALS

- Brucellosis infects many species, especially cattle, sheep, goats, pigs.
- Different *Brucella* types infect different species preferentially.
- Brucellosis presents typically as abortion in animals.
- Diagnosis can only be confirmed by laboratory tests.

Table 2 Animals affected by *Brucella* spp.

HOST	*B. abortus*	*B. melitensis*	*B. suis*	*B. canis*	*B. ovis*
Cattle	+	+	+(rare)	–	–
Buffaloes	+	+	–	–	–
Bison	+	–	–	–	–
Sheep	+(rare)	+	+(possible)	–	+
Goats	+(rare)	+	–	–	–
Swine	+(rare)	+(rare)	+	–	–
Dogs	+	+	+(rare)	+	–

HOST	*B. abortus*	*B. melitensis*	*B. suis*	*B. canis*	*B. ovis*
Camels	+(rare)	+	–	–	–
Caribou/Reindeer	–	–	+(biovar 4)	–	–
Elk	+	–	–	–	–
Horses	+	+(rare)	+(rare)	–	–
Rodents	+(rare)	+(rare)	+(biovar 5)	–	–

3. Epidemiology

3.1 Epidemiology of brucellosis in humans

3.1.1 Reservoirs of infection

Brucellosis is a zoonotic disease, hence the ultimate sources of infection are infected animals. The key species are the major food-producing animals: cattle, sheep, goats, pigs. Others, including bison, buffalo, camels, dogs, horses, reindeer and yaks are less important, but they can be very significant local sources of infection in some regions. Recently, the infection has also been identified in marine mammals, including dolphins, porpoises and seals, and these may present an emerging hazard to persons occupationally exposed to infected tissues from them.

The risk of disease and its severity is to a significant extent determined by the type of *Brucella* to which an individual is exposed. This will be influenced by the species of host animal acting as source of infection.

B. melitensis is the type most frequently reported as a cause of human disease and the most frequently isolated from cases. It is the most virulent type and associated with severe acute disease. It is recorded as endemic in several countries and accounts for a disproportionate amount of human brucellosis. The organism is normally associated with infection in sheep and goats, but other species, including dogs, cattle and camels can be infected. In some countries, particularly in the Middle East, *B. melitensis* infection of cattle has emerged as an important problem. Contrary to some traditional views, *B. melitensis* remains fully virulent for man after infecting cattle. The bovine infection presents a particularly serious problem because of the large volume of infected milk that can be produced by an individual animal and because of the extensive environmental contamination that even single abortions or infected births can produce.

B. abortus is the most widespread cause of infection, but associated with much less human disease. Infection in man is often sub-clinical and, where disease does occur, it is usually less severe than that caused by *B. melitensis* or *B. suis*. Cattle are by far the most common source of *B. abortus* but bison, buffalo, camels, dogs and yaks are important in some areas.

B. suis has a much more restricted occurrence than *B. melitensis* and *B. abortus*. It is locally important as a source of human infection which can be as severe as that produced by *B. melitensis*. The sources and virulence of the organism vary with its biovar (subtype defined by laboratory tests).

Biovars 1, 2, and 3 are associated with pigs and also, in the case of biovar 2, with hares. This variant has a low pathogenicity for humans but biovars 1 and 3 are highly virulent and can cause severe disease. Biovar 4 is associated with infection of caribou and reindeer in Alaska, Canada and Northern Russia. It is infrequently reported as a cause of human disease. Naturally acquired human cases of biovar 5 infection have not been reported.

B. canis is a widespread infection of dogs in many countries. It is infrequently associated with human disease. Reported cases have usually been mild.

Brucella infection occurs in many species of wild animals but these are rarely implicated as sources of human disease.

3.1.2 Transmission of brucellosis to humans

The possible means of acquisition of brucellosis include: person-to-person transmission, infection from a contaminated environment, occupational exposure usually resulting from direct contact with infected animals, and foodborne transmission.

3.1.2.1 Person-to-person transmission

This is extremely rare. Occasional cases have been reported in which circumstantial evidence suggests close personal or sexual contact as the route of transmission.

Of more potential significance is transmission through blood donation or tissue transplantation. Bone marrow transfer in particular carries a significant risk. It is advisable that blood and tissue donors be screened for evidence of brucellosis and positive reactors with a history of recent infection be excluded. Transmission to attendants of brucellosis patients is most unlikely but basic precautions should be taken. Laboratory workers processing samples from patients run a much greater risk.

3.1.2.2 Infection from a contaminated environment

This is difficult to document but probably occurs more frequently than is recognized. Infected animals passing through populated areas or kept in close proximity to housing may produce heavy contamination of streets, yards and market places, especially if abortions occur. Inhalation brucellosis may then result from exposure to contaminated dust, dried dung etc. Contact infection may also result from contamination of skin or conjunctivae from soiled surfaces. Water sources, such as wells, may also be contaminated by recently aborted animals or by run-off of rain water from contaminated areas.

Brucella spp. can survive for long periods in dust, dung, water, slurry, aborted fetuses, soil, meat and dairy products. The precise duration of survival is dependent on many variables such as the nature of the substrate, number of organisms, temperature, pH, sunlight, the presence of other microbial contaminants. Some examples are given in Table 3.

3.1.2.3 Occupational exposure

Certain occupations are associated with a high risk of infection with brucellosis. These include people who work with farm animals, especially cattle, sheep, goats and pigs: farmers, farm labourers, animal attendants, stockmen, shepherds, sheep shearers, goatherds, pig keepers, veterinarians and inseminators are at risk through direct contact with infected animals or through exposure to a heavily contaminated environment. Infection may occur by inhalation, conjunctival contamination, accidental ingestion, skin contamination especially via cuts or abrasions, and accidental self-inoculation with live vaccines.

The families of farmers and animal breeders may also be at risk as domestic exposure may be inseparable from occupational exposure when animals are kept in close proximity to living accommodation. In some areas, the animals are kept in the yards of houses and may even be brought inside, especially in severe weather. In the case of recently aborted animals, this has resulted in infection of entire households. The use of dried dung as a fuel may also import infection into households. It should be noted that brucellosis often presents as clusters of cases in a family or tribal group, usually relating to a common infected food source, and often follows an outbreak in animals.

Children can be particularly at risk as they may adopt newborn or sick animals as pets. In some areas they may be the only group presenting with acute symptoms, as older members of the community are likely to be immune or chronically infected.

Persons involved in the processing of animal products may be at high risk of exposure to brucellosis. These include slaughtermen, butchers, meat packers, collectors of fetal calf serum, processors of hides, skins and wool, renderers and dairy workers. Direct and environmental contamination may present hazards through inhalation, ingestion, mucous contamination and skin contact or penetration.

Staff employed in the maintenance of farm premises, factories or plants used for processing animal products are often overlooked as occupationally exposed groups but may be at considerable risk from environmental contamination.

Laboratory staff involved in culturing *Brucella* are at particular risk. In some countries in which brucellosis is no longer endemic, this potential hazard may be overlooked or considered no longer relevant. Nevertheless, the performance of diagnostic procedures on patients with unsuspected imported disease may lead to culture of organisms which are not correctly identified until laboratory-acquired infection raises the level of suspicion. The use of rapid identification gallery test systems has caused *Brucella* strains to be misidentified as *Moraxella* spp, with serious consequences for the staff. Inhalation of aerosols generated by manipulation of cultures presents the greatest hazard, especially if breakage of containers occurs during such processes as centrifugation.

The preparation and use of live vaccines is also hazardous as strains such as *B. abortus* S19 and *B. melitensis* Rev 1 are not completely avirulent for humans. The rough vaccine strain B. *abortus* RB 51 appears to be of low pathogenicity but still presents a potential hazard through accidental injection and is rifampicin-resistant. The use of virulent strains to prepare diagnostic antigens should also be avoided where possible.

3.1.2.4 Foodborne transmission

This is usually the main source of brucellosis for urban populations. Ingestion of fresh milk or dairy products prepared from unheated milk is the main source of infection for most populations. Cow, sheep, goat or camel milk contaminated with *B. melitensis* is particularly hazardous as it is drunk in fairly large volume and may contain large numbers of organisms. Butter, cream or ice-cream prepared from such milk also presents a high risk. Soft cheeses prepared from sheep or goats milk by addition of rennet are a particularly common source of infection in Mediterranean and Middle Eastern countries. The cheese-making process may actually concentrate the *Brucella* organisms, which can survive for up to several months in this type of product. Such cheeses should be stored in cool conditions for at least six months before consumption. Hard cheeses prepared by lactic and propionic fermentation present a much smaller risk. Similarly, yoghurt and sour milk are less hazardous. *Brucella* dies off fairly rapidly when the acidity drops below pH 4, and very rapidly below pH 3.5. Equipment used in the transport or processing of infected milk or other raw material may contaminate uninfected products unless good hygienic practice is observed.

Meat products are less frequently associated with infection, mainly because they are not usually eaten raw. However, this is a not unknown practice among butchers and abattoir workers. Muscle tissue usually contains low concentrations of *Brucella* organisms but liver, kidney, spleen, udder and testis may contain much higher concentrations. In some countries, dishes prepared from these organs may be eaten raw or undercooked. Fresh blood, either alone or mixed with fresh milk, may also be drunk and presents an obvious potential hazard.

In many countries, the consumption of “health foods” has become fashionable. These often include unpasteurized milk or milk products and may pose a particular risk. There is often considerable resistance to accepting that such “healthy” products can be dangerous. Raw vegetables may be contaminated by infected animals and present a hazard. In endemic areas, tourists consuming “ethnic” food products may be particularly at risk.

Persons with achlorhydria resulting from disease or through consumption of antacids or H2 antagonists may have an increased risk of acquiring brucellosis through ingestion of contaminated foods.

Individuals with immunodeficiency states resulting from disease or treatment with immunosuppressive agents may also be at increased risk of severe brucellosis, although this is difficult to quantify.

3.1.3 Seasonal factors

In countries with temperate or cold climates there is a marked seasonal variation in the incidence of acute brucellosis, with most cases occurring in the spring and summer. This coincides with the peak period for abortions and parturitions among farm animals and hence for the highest level of exposure of those attending the animals and consuming their milk. The seasonal effect is more obvious for ovine/caprine brucellosis than for bovine brucellosis, possibly because of the longer lactation period in cattle.

In tropical and subtropical areas, where animal breeding extends throughout the year, there is no seasonal influence on the incidence of brucellosis.

3.1.4 Age and sex distribution

In industrialized countries and in those others in which food hygiene prevents foodborne brucellosis, the disease is very largely occupational and the majority of cases are males between the ages of 20 and 45 years. In these situations, the disease is usually caused by *B. abortus or* B. *suis*. In countries or areas where *B. melitensis* is prevalent, the practices followed in marketing and distributing sheep and goat milk products in particular make the enforcement of hygienic measures very difficult. In this situation the whole population is at risk and many cases occur in women and children. In nomadic societies, the adults have often been exposed to infection at an early age and do not manifest acute disease, although many may have sequelae from chronic infection. Under such conditions children account for a high proportion of acute cases and brucellosis is largely a paediatric problem.

3.1.5 Travel-acquired brucellosis

Tourists or business travellers to endemic areas may acquire brucellosis, usually by consumption of unpasteurized milk or other dairy products. Travellers may also import infected cheeses or other dairy products into their own countries and infect their families or social contacts by this means. Imported cases now account for most of the acute brucellosis cases seen in North America and Northern Europe.

3.1.6 Bio-terrorism

B. melitensis and *B.suis* have been developed experimentally as biological weapons by state sponsored programmes. Their relative stability in aerosol form, combined with low infectious dose make them suitable agents for this purpose. *Brucella* could be used to attack human and/or animal populations. The impact is likely to be greatest in those areas in which the disease is not endemic. The organism can be obtained from natural sources in many parts of the world. Health and veterinary authorities should be aware of this potential source of infection.

Table 3 Survival periods of *B. abortus or B. melitensis* in various substrates.

Medium	Temperature or environment	Survival
B. abortus		
Solid surfaces	< 31 °C, sunlight	4–5 hours
Tap water	–4 °C	114 days
Lake water	37 °C, pH 7.5	< 1 day

Medium	Temperature or environment	Survival
Lake water	8 °C, pH 6.5	> 57 days
Soil – dried	~ 20 °C	< 4 days
Soil – wet	<10 °C	66 days
Manure	summer	1 day
Manure	winter	53 days
Farm slurry animal waste	ambient-temperature tank	7 weeks
Farm slurry animal waste	12 °C tank	> 8 months
B. melitensis		
Broth	pH > 5.5	> 4 weeks
Broth	pH 5	< 3 weeks
Broth	pH 4	1 day
Broth	pH < 4	< 1 day
Soft cheese	37 °C	48~72 hours
Yoghurt	37 °C	48~72 hours
Milk	37 °C	7~24 hours

KEY POINTS ON THE EPIDEMIOLOGY OF BRUCELLOSIS IN HUMANS

- Cattle, sheep, goats and pigs are the main reservoirs of *Brucella*.
- Transmission to humans occurs through occupational or environmental contact with infected animals or their products.
- Foodborne transmission is a major source of infection, with cheese made from raw milk and unpasteurized milk presenting a high risk.
- Brucellosis can be a travel-associated disease.
- Blood or organ/tissue transfer are possible sources of infection.
- Person-to-person transmission is extremely rare.

3.2 Epidemiology of brucellosis in animals

This will vary with the host species affected. For cattle, infection is usually caused by *B. abortus*. However, *B. melitensis* and rarely *B. suis* can also establish themselves in cattle and the mode of transmission is then similar to that for *B. abortus*. These infections are particularly dangerous to humans because of the high virulence of most *B. melitensis* and *B. suis* strains and of the large numbers of bacteria that are excreted by these animals.

In cattle and other Bovidae, *Brucella* is usually transmitted from animal to animal by contact following an abortion. Pasture or animal barn may be contaminated and the organisms are probably most frequently acquired by ingestion but inhalation, conjunctival inoculation, skin contamination and udder inoculation from infected milking cups are other possibilities. The use of pooled colostrums for feeding newborn calves may also transmit infection. Sexual transmission usually plays little role in the epidemiology of bovine brucellosis.

However, artificial insemination can transmit the disease and semen must only be collected from animals known to be free of infection.

In sheep and goats, *B. melitensis* is nearly always the infecting species. *B. ovis* can also infect sheep but is of little significance in relation to human disease. The mode of transmission of *B. melitensis* in sheep and goats is similar to that in cattle but sexual transmission probably plays a greater role. The transmission of disease is facilitated by commingling of flocks and herds belonging to different owners and by purchasing animals from unscreened sources. The sharing of male breeding stock also promotes transfer of infection between farms. Transhumance of summer grazing is a significant promoting factor in some areas as is the mingling of animals at markets or fairs. In cold climates, it can be the custom to house animals in close space and this also facilitates transmission of infection.

Swine brucellosis is transmitted by direct contact with recently aborted sows, by ingestion of contaminated food or exposure to a contaminated environment. However, sexual transmission is particularly important. Brucellosis may be introduced on to farms through the communal use of boars or by purchase of infected animals.

For all species, embryo transfer is safe provided that recommended procedures are followed.

B. canis can be a major problem in dog breeding kennels. Transmission is by contact with recently aborted animals or with food or environment contaminated by abortions or excreta. Sexual transmission is also an important means of spread and males can excrete the organisms in large numbers in their semen. Urinary excretion also occurs and is a potential hazard to humans. However, in some countries where *B. canis* is present in the dog population, overt human disease caused by this organism seems to occur infrequently.

It should be remembered that dogs can acquire infection with *B. abortus*, *B. melitensis* or *B. suis* from aborted ruminants or swine, usually by ingesting fetal or placental material. They can then excrete these bacteria and may present a serious hazard to humans and domestic livestock.

B. suis biovar 4 causes brucellosis in caribou and reindeer. The epidemiology is similar to that of bovine brucellosis. Transmission to people can occur through the usual routes. However, ingestion of raw or undercooked reindeer bone marrow has also been implicated as a source of human infection.

In cattle, sheep, goats and swine, susceptibility to brucellosis is greatest in sexually mature animals. Young animals are often resistant, although it should be noted that latent infections can occur and such animals may present a hazard when mature.

Breed may also affect susceptibility, particularly in sheep. The milking breeds seem to be the most susceptible to *B. melitensis*. Breed differences in susceptibility have not been clearly documented in cattle although genetically determined differences in susceptibility of individual animals have been demonstrated. Polymorphism of the natural resistance associated monocyte protein (NRAMP) gene has been shown to influence substantially susceptibility to brucellosis in cattle and pigs. However, management practices are far more important in determining the risk of infection. Latent or inapparent infections can occur in all farm animal species. These usually result from infection in utero or in the early post-natal period. Such animals can retain the infection for life and may remain serologically negative until after the first abortion or parturition. Latent infection has been estimated to occur in the progeny of about 5% of infected cows. The extent of the problem in other species is not known, but latency has been documented in sheep.

Acquired immunity has a substantial effect on susceptibility. Vaccination of cattle with *B. abortus* strain 19 or RB 51, or sheep and goats with *B. melitensis* Rev 1 can reduce susceptibility a thousand fold or more to the homologous species. *B. abortus* strain 19 does not protect cattle against *B. melitensis*. However, there is little information on the use of Rev 1 vaccine in cattle. The efficacy of this vaccine against the *B. melitensis* strains

prevalent in some areas has also been questioned. Vaccines must be obtained from a reliable, internationally approved source. It is possible that strains of *B. melitensis* exist which can circumvent the immunity induced by this vaccine. However, it is at least as probable that variations in vaccine quality have affected protection rates. For the present, Rev 1 vaccine is the most effective vaccine available against *B. melitensis* and in many countries has given very good results. Its use is recommended when uncontrolled *B. melitensis* infection exists in ruminant populations.

KEY POINTS ON THE EPIDEMIOLOGY OF BRUCELLOSIS IN ANIMALS

- *B. abortus* causes most brucellosis in cattle, but *B. melitensis* and *B. suis* can also cause bovine infection.
- *B. melitensis* is the main cause of brucellosis in sheep and goats and *B. suis* in swine.
- Transmission occurs by direct contact and environmental contamination following abortion.
- Sexual transmission and/or artificial insemination are also important.
- Seronegative latent infections can occur.

4. Diagnosis

The isolation and identification of *Brucella* offers a definitive diagnosis of brucellosis and may be useful for epidemiological purposes and to monitor the progress of a vaccination programme in animals. *Brucella* represents a risk to personnel handling it in the laboratory. Attention must be paid to the local legal requirements for handling *Brucella* and it is essential that certain minimum standards of laboratory safety are adhered to. These are specified in Section 6. For information on the international standards of diagnostic tests, please also refer to the *Manual of diagnostic tests and vaccines for terrestrial animals*, OIE, 2004

4.1 Diagnosis in humans

The diagnosis of human brucellosis cannot be made solely on clinical grounds due to the wide variety of clinical manifestations of this disease, and it is essential to perform bacteriological and serological tests. However, all physicians dealing with a febrile patient living in an endemic area or recently travelled to a country where brucellosis is endemic ("travel-associated disease") must be aware of the possibility that the patient could be infected with *Brucella*. For this reason, correct clinical history taking is essential to orientate the diagnosis, and the need for some very basic questions (profession, food ingested, contact with animals and travel to endemic areas) must be emphasized. Moreover, a rapid screening test must be performed. The Rose Bengal plate test can be used as a sensitive rapid screening test but the results should be confirmed by bacteriological and other serological tests. Should the screening test prove negative in the face of a history and clinical presentation, it is advisable to check the result using additional tests. Careful observance of these practices will help to avoid delayed diagnosis.

4.1.1 Bacteriological diagnosis

The only conclusive evidence of *Brucella* infection is the recovery of the bacteria from the patient. Although *Brucella* can be isolated from bone marrow, cerebrospinal fluid, wounds, pus, etc., blood is the material most frequently used for bacteriological culture. Concentrating and lysing the leukocyte fraction before culture is reported to improve the isolation rate.

The system of blood culture that is recommended is the biphasic method of Castaneda which uses both solid and liquid medium in the same container. This limits the need for subculture and thus reduces the risk of laboratoryacquired infection. Serum dextrose broth with corresponding solid phase is often recommended but *Brucella* will grow on most high quality peptone based media used for blood culture. Selective medium is not necessary for culture of human blood samples taken with aseptic precautions. Incubation should be performed in air supplemented with 5% CO_2. The newer semiautomatic methods (BACTEC 9204 and Bac/Alert) shorten considerably the time taken for detection; the presence of *Brucella* can be detected with these methods by the third day of incubation. However, limited published data exist for a significant number of blood cultures that would permit comparison of these and traditional methods. It should be noted that earlier systems (BACTEC NR 730) failed to detect an appreciable number of samples which were positive by conventional blood culture systems.

Conventional Castaneda blood cultures are seldom positive before the fourth day of incubation. The majority of blood cultures are positive between the seventh and 21st day, and only 2% are positive after the 27th day. For this reason, incubations should be carried out for at least 45 days before rejecting a blood culture as negative for *Brucella*. Periodic tipping of the broth-blood mixture in Castaneda bottles over the solid phase should minimise the need for subculture. However, if no growth has appeared after one week it is recommended that the liquid be subcultured on to a solid medium, and that smears be prepared and stained with the modified Ziehl-Neelsen method of Stamp. This process can distinguish *Brucella* from the possible Gram positive cocci and bacilli skin contaminants, and from artifacts present in media inoculated with blood.

In Mediterranean countries, for unknown reasons, *B. abortus* is rarely isolated from human cases. In Spain, for example, over 98% of 2107 isolates from humans examined in one study were *B. melitensis*. The percentage of positive blood cultures in patients with fever can be as high as 86.5%. In patients either with low fever or without fever, the percentages fall to 75% and 28.5% respectively. This also holds true for relapsed patients. However, it has been shown in two extensive studies that 31.8% and 41.9% isolations of *B. melitensis* were from patients without fever.

A presumptive identification of *Brucella* isolates at genus level can be made on the basis of colonial morphology, appearance of smears stained with the methods of Gram and Stamp, and the results of oxidase and slide agglutination tests with *Brucella-specific* antisera. Alternatively, if a validated molecular identification method is available, such as PCR with primers for the genusspecific insertion sequences *IS 711* or IS*650*, or sections of the *16S-23S rRNA, BCPS31* and *omp 2a* genes, this may be used. The presumptive *Brucella* isolate should be submitted to a reference laboratory for a precise identification at species and biovar level, as this can provide very valuable epidemiological information.
Recently, some authors have proposed PCR-based assays for the direct detection of *Brucella* organisms in blood. However, more experience is needed before deciding whether this can replace the traditional blood cultures. Before any such assay is introduced into the routine laboratory tests, it must be validated for sensitivity, specificity and reproducibility. Before implementing any such procedures, it is essential to institute efficient containment procedures to prevent contamination of samples with bacterial DNA or amplified replicons from the laboratory environment.

4.1.2 Serological diagnosis

4.1.2.1 Antigens and immunoglobulins of diagnostic significance

The major *Brucella* antigens that are useful for diagnosing human brucellosis are the smooth (S) lipopolysaccharide (LPS) of the outer cell membrane and the internal (cytosolic) proteins. As in the case of other Gram-negative bacteria in the S-phase, the surface of *Brucella* is an outer membrane containing SLPS which is exposed to the environment. The LPS is the immunodominant antigen but is also the molecule carrying the epitopes that may cross-react with other Gram-negative bacteria including *Yersinia enterocolitica* O:9, *Escherichia coli* O:157, *Francisella tularensis, Salmonella urbana* O:30, *Vibrio cholerae, and others*.

The serum (tube) agglutination test (SAT), or micro-titre plate variants of this, using heat/phenol-killed whole S-cells, detects antibodies to the S-LPS. Antibodies reacting against S-LPS can also be detected by other tests – e.g. enzyme-linked immunosorbent assay (ELISA) – when they are adapted to use extracts which contain S-LPS. Since this is the immunodominant antigen, antibodies to proteins are detected using S-LPS-free cytosolic protein preparations. An important point in the use of the cytosolic proteins is that a significant serological cross-reactivity with the above mentioned bacteria has never been found (although cross-reactivity with bacteria such as *Ochrobactrum* that are closely related genetically to *Brucella* is possible). Therefore, these proteins can be used to distinguish infections caused by *Brucella* from those caused by bacteria cross-reacting at S-LPS level.

The human immune response to *Brucella* is characterized by an initial production of IgM isotype antibodies followed after a longer period by the secretion of IgG isotype antibodies. The ELISA using S-LPS can be used to measure the evolution of immunoglobulin isotypes following infection and after treatment. A good correlation has been found between ELISA-IgM and serum agglutination titres. In one study, after treatment the titre of IgM antibodies appeared to decline faster than that of IgG antibodies. However, between 25% and 50% of patients with acute brucellosis presented IgM antibodies one year after treatment. Among these patients, 85% had high titres of IgG 18 months after clinical recovery, and in patients suffering a relapse, there was a concomitant increase in IgG but not IgM. IgA titres roughly paralleled IgG titres. In contrast, in a different study using SAT and 2-mercaptoethanol (2-ME) or dithiothreitol (DTT) agglutination, patients successfully treated for brucellosis had a rapid decline in 2-ME resistant (IgG) and sensitive (IgM) agglutinins. However, low levels of 2-ME sensitive (IgM or IgA) agglutinins, as measured by SAT, could remain in the serum for long periods of time. It should be noted that reduction tests are not totally specific for IgM as they can degrade IgA as well. Therefore their results must be interpreted with caution. ELISA is to be preferred for detecting specific isotypes. The apparently different results of the 2-ME and ELISA-IgG are partly due to this effect and partly to the detection in the latter test of small amounts of antibodies of the IgG class that do not agglutinate. Since it is important to correlate the titres of antibody to *Brucella* with the clinical course of infection, one must be aware of the antibody class that is measured by the individual tests.

Theoretically, in acute brucellosis, the first and principal immunoglobulin isotype is IgM. Subsequently there is a switch to IgG isotype synthesis in patients who have not received treatment. The initial IgM response may not be seen in patients with a slow insidious onset of disease, in those seen late in the course of the disease or in those with relapses. The titres of agglutinins (IgM, IgA and IgG), should decline after successful treatment; if they do not, it is necessary to evaluate the patient for the possibility of a relapse or chronic focal disease. IgG and IgA titres increase in relapses.

4.1.2.2 Serological tests that detect antibodies against S-LPS

The RBT is currently the recommended rapid screening test, but the results should always be confirmed by other tests detecting agglutinating and nonagglutinating antibody and by bacteriological culture, particularly in areas where there is a high incidence of animal brucellosis. The sensitivity of RBT is over 99%, but it can give false positive reactions with sera from patients infected with *Y. enterocolitica* 0:9 or other cross reactive organisms and from healthy individuals that have had contact with *S-Brucella* without developing disease.

The SAT is a very useful test for the diagnosis of human brucellosis when it is performed with a standardized antigen preparation, and titres which can be expressed in International Units (IU) can be correlated well with clinical stages of infection. To make this test more informative, an agglutination in parallel should be performed using as diluent phosphate buffer containing 2-ME at a final concentration of 0.05 M or DTT at a final concentration of 0.005 M, which destroys the agglutinating activity of IgM (and IgA).

The problem of defining an SAT titre indicative of active infection has yet to be solved. In general, each patient produces an individual response and it is not possible to predict the behaviour of this in each case, nor to explain why some patients develop high agglutinin titres, while others have only low values during the disease. For example, in a study of 238 brucellosis patients, it was found that, if a titre of 1/80 (100 IU) was to

be considered as diagnostic, 29.2% of the patients would be considered negative; furthermore, in the same study it was found that 3.4% of the sera had a titre of 1/10 (12 IU) or below. As a guide, titres of 160 (200 IU) or more have a clear diagnostic value as long as the patient presents signs and symptoms of the disease. Some authors consider that in areas with a high incidence of animal brucellosis the diagnostic titre should be higher than this as many asymptomatic individuals will have titres at this level. In these circumstances the value of the SAT is severely limited. Although seroconversion is highly indicative of infection it is seldom seen in practice, because serum samples are rarely taken at a sufficiently early stage of infection.

A good correlation between the results of IgG ELISA and Coombs tests has been reported. However, the Coombs test (and IgG ELISA) remains positive longer than other agglutination tests. The titres of Coombs tests are usually very high when infection with *Brucella* has been present for a long time before the diagnosis is made. This may be summarized as follows: in acute brucellosis, Coombs titres are usually 4 to 16 times higher than SAT titres, whereas in patients with a long period of evolution without treatment the titres are 16 to 256 times higher.

Although immunofluorescence and radioimmunoassay have also been used in the past, the most suitable method for studying the immunoglobulin isotype distribution is the indirect ELISA. The analysis of data shows that ELISA is useful for measuring IgG antibodies and that it is possible to replace the Coombs test by an indirect ELISA with S-LPS and anti-IgG conjugates. However, although ELISA with S-LPS is a very promising test, several problems, including standardization, variable quality of commercial reagents and interpretation of results, particularly when based on optical density readings alone, cause problems of inter-laboratory comparability and need to be resolved by the establishment of standard reference materials.

The routine use of the complement fixation test (CF) is not recommended in small laboratories because of its technical complexity (much greater than that of SAT and ELISA) and because of the problems encountered in its standardization. However, this test is useful. Experience has shown that: (i) the CF and SAT are positive in 91.7% of cases; (ii) CF titres are higher than SAT titres after the 4th or 5th month of illness; (iii) a negative CF result with a significant SAT titre occurs in approximately 4.6% of patients, generally in the initial days or weeks of illness; and (iv) a negative SAT result with high CF titres occurs in approximately 3.7% of cases, generally corresponding to chronic illness or to patients who have recovered.

4.1.2.3 Serological tests that detect antibodies against cytosolic proteins

Antibodies to cytosolic protein antigens of *Brucella* have been studied by counter-immunoelectrophoresis (CIEP), ELISA and western blotting. By CIEP it has been found that sera of those patients with 2-ME resistant antibodies and high Coombs titres also produce a greater number of precipitation lines and higher titres of antibodies to proteins. Obviously, the responses of those patients had longer evolution times and this is interpreted to mean that the number of precipitation lines increases as the disease evolves without diagnosis and treatment. The ELISA studies have shown that, while antibodies to S-LPS may appear in persons that have had contact with S-*Brucella* but have not developed clinical disease, the antibodies to selected proteins are indicative of an active infection. In a series of patients with persistent infection or relapse it was found that titres of anti-protein antibodies remained elevated, whereas in patients who recovered anti-protein antibodies disappeared. Qualitatively similar results have been obtained with western blotting. The shortcomings of these methods include their non-quantitative and rather subjective interpretation, the lack of validation data, and the non-availability of reference reagents.

4.1.3 Diagnosis of *Brucella* meningitis and meningoencephalitis

In such cases, ideally, *Brucella* should be cultured from CSF. However, bearing in mind that in most cases the routine cultures give negative results, it is mandatory to perform serological tests on CSF. In brucellosis that is not affecting the central nervous system, patients do not develop antibodies in the CSF. In contrast, in those patients in whom neurobrucellosis develops, the CFS contains low titres of antibodies against S-LPS and

cytosolic proteins. These antibodies can be easily detected by RBT and CIEP tests respectively. The CSF is usually clear and its analysis reveals an increase of IgG and a lymphocytic pleocytosis.

4.1.4 Intradermal tests

The development of delayed hypersensitivity to intradermally administered *Brucella*-specific antigens is an indication of past exposure to infection but does not indicate its current significance. Although used in the past in some countries, the intradermal test is not recommended for diagnosis. The use of undefined and unstandardized antigen preparations may also provoke antibodies which interfere with subsequent serological tests.

4.1.5 Conclusion

A correct serological diagnosis of human brucellosis can be made with a test that uses S phase, whole cells. Recommended tests are RBT, SAT alone or with 2-ME or DTT reduction, Coombs antiglobulin, CFT and ELISA. The results of a combination of tests such as SAT and Coombs antiglobulin can be used to assess the stage of evolution of the disease at the time of diagnosis. The ELISA, with a conjugate of the appropriate IgM or IgG specificity and S-LPS, could replace established tests but requires further standardization and validation. Other methods can be useful but are less specific and have not been adequately evaluated.

KEY POINTS ON THE DIAGNOSIS OF BRUCELLOSIS IN HUMANS

- In acute brucellosis, isolation of *Brucella* from blood or other tissues is definitive.
- Culture is often negative, especially in long-standing disease.
- Serology is the most generally useful diagnostic procedure approach.
- The RBT, tube agglutination and ELISA procedures are recommended.
- Methods which differentiate IgM and IgG can distinguish active and past infection.
- False positive serological reactions may occur.
- Skin test reactions indicate past exposure not active infection.

4.2 Diagnosis in animals

Diagnosis and control of the disease in animals must be carried out on a herd basis. There may be a very long incubation period in some infected animals and individuals may remain serologically negative for a considerable period following infection. The identification of one or more infected animals is sufficient evidence that infection is present in the herd, and that other serologically negative animals may be incubating the disease and present a risk.

Diagnostic tests fall into two categories: those that demonstrate the presence of the organisms and those that detect an immune response to its antigens. The isolation of *Brucella* is definitive proof that the animal is infected, but not all infected animals give a positive culture and the methods and facilities that must be employed are not always readily available. The detection of antibody or a hypersensitivity reaction provides only a provisional diagnosis, but in practice is the most feasible and economic means of diagnosis. False positive reactions to serological tests can occur through a number of factors, including vaccination, and this must be borne in mind when interpreting results. Similarly, dermal hypersensitivity only indicates previous exposure to the organism, not necessarily active infection, and may also result from vaccination.

Vaccination is an extremely important and effective facet of most control strategies but has the disadvantage that its use may confuse diagnosis by stimulating the production of hypersensitivity or antibodies detectable by serological tests. Antibody titres may persist for a prolonged period in a small proportion of vaccinated animals and this proportion increases with age at vaccination. To reduce this problem, in cattle vaccination is usually employed in young animals below the age of six months, but may be used in adults if a reduced dose is given, especially by the intraconjunctival route. There is currently no widely available test that is able to distinguish vaccinated from infected animals, although some tests are under evaluation.

It is of utmost importance that the use of vaccination is strictly controlled, that it is used at the correct age, that vaccine of sufficient quality is used and that vaccinated animals are correctly identified. If this is not the case, correct serological diagnosis is confused. The vaccination programme can be suspended when the prevalence of the disease reaches a very low level, when the disadvantages of vaccination outweigh any benefit that it may bring on the basis of cost-benefit and cost-effectiveness analysis.

4.2.1 Bacteriological methods

The isolation and identification of *Brucella* offers a definitive diagnosis of brucellosis and may be useful for epidemiological purposes and to monitor the progress of a vaccination programme. It should be noted that all infected materials present a serious hazard, and they must be handled with adequate precautions during collection, transport and processing.

4.2.1.1 Stained smears

Smears of placental cotyledon, vaginal discharge or fetal stomach contents may be stained using modified Ziehl-Neelsen (Stamp) or Kosters' methods. The presence of large aggregates of intracellular, weakly acid-fast organisms with *Brucella* morphology is presumptive evidence of brucellosis. Care must be taken as other infectious agents such as *Coxiella burnetii* or *Chlamydia* may superficially resemble *Brucella* (Fig. 3).

4.2.1.2 Culture

Brucella may most readily be isolated in the period following an infected abortion or calving, but isolation can also be attempted post-mortem.

Brucella are excreted in large numbers at parturition and can be cultured from a range of material including vaginal mucus, placenta, fetal stomach contents and milk using suitable selective culture media. It is of the utmost importance that faecal and environmental contamination of the material is kept to a minimum to give the greatest chance of successfully isolating *Brucella.* If other material is unavailable or grossly contaminated, the contents of the fetal stomach will usually be otherwise sterile and are an excellent source of *Brucella*.

In some circumstances it may be appropriate to attempt the isolation of *Brucella* post-mortem. Suitable material includes supramammary, internal iliac and retropharyngeal lymph nodes, udder tissue, testes and gravid uterus.

Milk samples should be allowed to stand overnight at 4 °C before lightly centrifuging. The cream and the deposit are spread on to the surface of at least three plates of solid selective medium. Placental samples should be prepared in the field by selecting the least contaminated portion and cutting off pieces of cotyledon. In the laboratory, the portions should be immersed in alcohol which should be flamed off before cutting with scissors or scalpel and smearing the cut surface on three plates of selective medium. Other solid tissues can be treated in a similar manner, or, ideally, they should be macerated mechanically following flaming before plating out. The tissues may be ground manually or homogenised in a blender or stomacher with a small proportion of sterile water. Fetal stomach contents are collected, after opening the abdomen, by searing the surface of the stomach with a hot spatula and aspirating the liquid contents with a Pasteur pipette or syringe.

Bacterial colonies may be provisionally identified as *Brucella* on the basis of their cultural properties and appearance, Gram staining, and agglutination with positive antiserum (Fig. 4 and 5). If available, a PCR-based molecular identification method may be used. Definitive identification of suspect colonies can only be made using techniques available at *Brucella* Reference Centres.

4.2.2 Serological methods

The detection of specific antibody in serum or milk remains the most practical means of diagnosis of brucellosis. The most efficient and cost-effective method is usually screening all samples using a cheap and rapid test which is sensitive enough to detect a high proportion of infected animals. Samples positive to screening are then tested using more sophisticated, specific confirmatory tests for the final diagnosis to be made (Fig. 5 and 6).

It is absolutely essential that only internationally recognized tests using antigens standardized against the 2nd International anti-*B. abortus* Serum are used. Appropriate quality control sera should be included with each batch of tests, and tests should be repeated if the quality control criteria are not met.

Serological results must be interpreted against the background of disease incidence, use of vaccination and the occurrence of false positive reactions due to infection with other organisms. As with all laboratory based diagnosis, it is imperative to correctly identify the “audit trail” of individual animal identity, sample number and test result so that there is complete certainty of the linkage between animal and result.

4.2.2.1 Rose Bengal plate test (RBT)

The RBT is one of a group of tests known as the buffered *Brucella* antigen tests which rely on the principle that the ability of IgM antibodies to bind to antigen is markedly reduced at a low pH. The RBT and other tests such as the buffered plate agglutination tests and the card test play a major role in the serological diagnosis of brucellosis worldwide (Fig. 7).

The RBT is a simple spot agglutination test where drops of stained antigen and serum are mixed on a plate and any resulting agglutination signifies a positive reaction. The test is an excellent screening test but may be oversensitive for diagnosis in individual animals, particularly vaccinated ones. The procedure can be automated but this requires custom-made equipment.

4.2.2.2 ELISA tests

The ELISA tests offer excellent sensitivity and specificity whilst being robust, fairly simple to perform with a minimum of equipment and readily available from a number of commercial sources in kit form. They are more suitable than the CFT for use in smaller laboratories and ELISA technology is now used for diagnosis of a wide range of animal and human diseases. Although in principle ELISAs can be used for the tests of serum from all species of animal and man, results may vary between laboratories depending on the exact methodology used. Not all standardization issues have yet been fully addressed. For screening, the test is generally carried out at a single dilution. It should be noted, however, that although the ELISAs are more sensitive than the RBT, sometimes they do not detect infected animals which are RBT positive. It is also important to note that ELISAs are only marginally more specific than RBT or CFT.

4.2.2.3 Serum agglutination test (SAT)

The SAT has been used extensively for brucellosis diagnosis and, although simple and cheap to perform, its lack of sensitivity and specificity mean that it should only be used in the absence of alternative techniques.

4.2.2.4 Complement fixation test (CFT)

The sensitivity and specificity of the CFT is good, but it is a complex method to perform requiring good

laboratory facilities and trained staff. If these are available and the test is carried out regularly with good attention to quality assurance, then it can be very satisfactory.
It is essential to titrate each serum sample because of the occurrence of the prozone phenomenon whereby low dilutions of some sera from infected animals do not fix complement. This is due to the presence of high levels of non-complement fixing antibody isotypes competing for binding to the antigen. At higher dilutions these are diluted out and complement is fixed. Such positive samples will be missed if they are only screened at a single dilution.

In other cases, contaminating bacteria or other factors in serum samples fix or destroy complement causing a positive reaction in the test, even in the absence of antigen. Such "anti-complementary" reactions make the test void and a CFT result cannot be obtained.

4.2.3 Supplementary tests

Many other serological tests have been employed. Some, such as the Rivanol or 2-ME test, are variations of the SAT and, although more specific, share many of its disadvantages. At present, the use of such procedures in the place of the standard test is not advised.

4.2.3.1 Milk testing

In dairy herds, milk is an ideal medium to test as it is readily and cheaply obtained, tests can be repeated regularly and give a good reflection of serum antibody. Milk from churns or the bulk tank can be screened to detect the presence of infected animals within the herd which can then be identified by blood testing. This method of screening is extremely effective and is usually the method of choice in dairy herds.

4.2.3.2 Milk ring test

The milk ring test (MRT) is a simple and effective method, but can only be used with cow's milk. A drop of haematoxylin-stained antigen is mixed with a small volume of milk in a glass or plastic tube. If specific antibody is present in the milk it will bind to the antigen and rise with the cream to form a blue ring at the top of the column of milk. The test is reasonably sensitive but may fail to detect a small number of infected animals within a large herd. Non-specific reactions are common with this test, especially in brucellosisfree areas. The milk ELISA is far more specific than the MRT.

4.2.3.3 Milk ELISA

The ELISA may be used to test bulk milk and is extremely sensitive and specific, enabling the detection of single infected animals in large herds in most circumstances.

4.2.3.4 Fluorescence polarization assay

This technique, which requires special reagents and reading equipment, is claimed to have advantages in sensitivity and specificity over other methods. Evaluation has been limited however, and the procedure is not widely available. Further information is required before its overall value can be assessed.

4.2.3.5 Intradermal test

This procedure, using a standardized antigen preparation such as Brucellin INRA or Brucellergene OCB, can be used for monitoring the status of herds in brucellosis-free areas. It is sensitive and specific but false positive reactions can occur in vaccinated animals.

4.3 Remarks on the diagnosis of brucellosis other than cattle

The procedures described above are primarily intended for the diagnosis of brucellosis in cattle. However, the bacteriological methods are also applicable to the diagnosis in all other species. The serological procedures

require some modification for individual species as follows:

4.3.1 Sheep and goats

The RBT is useful for screening sheep and goat sera for antibodies to *B. melitensis*. An antigen suspension adjusted for highest sensitivity against a panel of control sera is recommended. The test is less sensitive than in cattle, however, and may not detect some infected animals. It is best used in combination with the complement fixation test.

The SAT using 5% sodium chloride as diluent has been widely used but has low sensitivity and specifity.

The micro-agglutination variant has higher sensitivity and specificity. It is only recommended in situations when more sophisticated tests are not available. The antigen is standardized to give 50% agglutination with a 1:650 dilution of the second International Standard for *B. abortus* antiserum.

It should be noted that agglutination methods are particularly sensitive to non-specific agglutinins and cross-reacting antibodies. They tend to be more sensitive in the acute phases of infection and are severely affected by vaccination. They should only be used if no alternative is available. The CFT is superior to agglutination methods but its sensitivity and specificity are limited and it should be regarded as a complementary rather than confirmatory test. It may be used for screening if automated methods are available. Sera should be inactivated at 62 °C for 30 min. Vaccination produces seroconversion in the CFT but, in that case, antibody titres decline much more rapidly than those resulting from infection. ELISA is reported to give superior results to other tests in sensitivity and specificity, but experience is limited. The MRT is not suitable for use on sheep or goat milk but ELISA can be used. The CFT is also usable on whey samples but is technically demanding and no longer recommended. It should be understood that no currently available serological test can be considered reliable for the detection of ovine or caprine brucellosis at the level of individual animals. Diagnosis and control should be applied at the herd/flock level.

The intradermal test for delayed hypersensitivity to *Brucella* antigens is useful as a flock or herd test. However, it is affected by vaccination. A purified antigen preparation which contains a mixture of *Brucella* proteins free of smooth LPS, should be used to avoid compromising serological tests. It is useful for monitoring the status of brucellosis-free flocks, especially if vaccination is not practised.

4.3.2 Pigs

Serological tests are much less satisfactory for detecting pigs with brucellosis than for diagnosis of the disease in cattle, sheep and goats. Testing should be done on a herd basis. Non-specific agglutinins and cross-reacting antibodies engendered by intercurrent infections with *Escherichia coli* O157, *Salmonella*, *Yersinia enterocolitica* 09 and other organisms are common. The RBT is useful for screening large numbers of sera. The SAT is not recommended. The CFT gives results comparable with the RBT. ELISA offers the highest sensitivity and specificity of all currently available serological tests.

The intradermal test, using a defined antigen preparation is the most reliable diagnostic procedure for pigs on both an individual or herd basis. When infection is detected, it should be dealt with by slaughter of the herd as many infected animals are likely to remain undetected.

4.3.3 Camels, buffalo, reindeer, yaks

The serological tests used for cattle are applicable to these species. Camel sera for testing in the CFT should be inactivated at 60~62 °C for 30 min. The Rivanol test has been recommended for examining buffalo sera. The ELISA has not been widely evaluated for most of these species but is potentially useful subject to adequate standardization.

4.3.4 Dogs

B. abortus, *B. melitensis,* and *B. suis* infection in dogs can be diagnosed using the procedures described for cattle, except for ELISA, which has not been widely assessed in dogs. For *B. canis* infection the most reliable procedure is isolation of the organisms. As persistent bacteraemia is common, blood culture is a useful procedure. Serological tests are less satisfactory. They must use antigens prepared from *B. canis* or *B. ovis* strains as the surface antigens of smooth *Brucella* spp. do not cross-react with these. ELISA is probably the most useful procedure but is not widely available.

KEY POINTS ON THE DIAGNOSIS IN ANIMALS

- Culture of *Brucella* from abortion material, milk or tissues collected at autopsy provides a definitive diagnosis.
- Serology is usually the most practicable method.
- Cattle: the RBT is recommended for screening; ELISA or complement fixation are recommended for confirmation of infection in individual animals. Screening of milk samples by milk ring test or ELISA is useful for surveillance.
- Sheep, goats and pigs: no single serological test is reliable for confirmation of infection in individual animals. Serological tests should be used on a herd or flock basis. Similarly, the skin test is useful for screening at the herd or flock level, especially if vaccination is not used.
- A “rough-specific” antigen must be used for *B. canis* serology

5. Treatment of human brucellosis

The essential element in the treatment of all forms of human brucellosis is the administration of effective antibiotics for an adequate length of time. This should be within the context of general medical supervision and, for severely ill patients, is best carried out in hospital if circumstances permit. Antibiotic treatment should be implemented at as early a stage as possible, even in patients who appear to be showing a spontaneous improvement. In those patients with complications, additional treatment, including in some cases surgical intervention, will be necessary.

Uncomplicated acute brucellosis almost invariably responds well to appropriate antibiotic treatment. Patients and their families should be reassured that full clinical and bacteriological recovery is usual in human brucellosis.

A variety of antimicrobial drugs have activity in vitro against *Brucella* species; however, the results of routine susceptibility tests do not always correlate with clinical efficacy. Consequently, beta-lactam antibiotics, such as penicillins and cephalosporins, and macrolide antibiotics, such as erythromycin, are associated with unacceptably high rates of relapse when used to treat patients with brucellosis. Although newer macrolides, such as azithromycin and clarithromycin are more active in vitro than erythromycin, they have not shown superiority over current regimens for treatment of patients with brucellosis, and their role in therapy remains to be determined.

5.1 Treatment of uncomplicated brucellosis in adults and children eight years of age and older

5.1.1 Tetracyclines

Tetracycline (500 mg every six hours orally) administered for at least six weeks has long been the standard treatment of human brucellosis. Doxycycline (a long acting tetracycline analogue) is now the preferred drug because it can be given once or twice daily, and is associated with fewer gastrointestinal side effects than tetracycline. Doxycycline is given in a dose of 100 mg every 12 hours orally and is administered for a period of six weeks.

5.1.2 Aminoglycosides

Because the rate of relapse when tetracycline or doxycycline are given alone remains between 10%~20%, most authorities recommend an amino-glycoside to be given in addition to the tetracyclines for the first two to three weeks of therapy.

Streptomycin (1 g/day intramuscularly) administered for two to three weeks has long been the aminoglycoside of choice when used in combination with tetracycline or doxycycline. Although synergy between the two drugs is difficult to prove using routine in vitro assays, bacterial killing studies have shown that *Brucella species* undergo a more rapid rate of killing by the combination than by either drug alone.

Gentamicin is more active in vitro against *Brucella* species than streptomycin and, when administered as a single daily dose, is associated with few adverse side-effects. Although gentamicin, in a dose of *5mg/* (*kg/day*) intravenously or intramuscularly, administered for 7 to 10 days in combination with doxycycline administered for six weeks, yielded good results in one study, experience with this regimen is too limited to justify its use over doxycycline plus streptomycin. Unfortunately, no direct study comparing the results of doxycycline plus streptomycin versus doxycycline plus gentamicin has yet been published. Until additional experience is gained using gentamicin in place of streptomycin, the optimal dose and duration of therapy remain unknown.

5.2 Principal alternative therapy

Rifampicin is active in vitro against *Brucella* species, is remarkably lipid soluble, and it accumulates within eukaryotic cells. In order to provide a completely oral regimen with which to treat brucellosis, the combination of doxycycline (200 mg/day orally) plus rifampicin (600~900 mg/day orally), with both drugs administered for six weeks, was recommended by the WHO Expert Committee in 1986. This regimen has generally been found to be of similar efficacy to doxycycline plus streptomycin for patients with uncomplicated brucellosis. Caution is advised when considering this regimen for patients with complications, such as spondylitis. An analysis of various treatment regimens concluded that overall the regimen of doxycycline plus streptomycin was likely to be the most effective. In addition, some data have been reported indicating that rifampicin might enhance the plasma clearance of doxycycline, thus yielding subtherapeutic levels – a possible explanation of treatment failures with this regimen.

5.3 Secondary alternative therapy

Fluoroquinolones. Fluoroquinolone antibiotics have greater activity in vitro against *Brucella* species than the parent drug nalidixic acid. In addition, they are well absorbed after oral administration, and they achieve high concentrations within phagocytic cells. Although the minimum bactericidal concentration of quinolones is reported to be approximately four times the minimum inhibitory concentration, a lack

of bactericidal activity was found at pH levels comparable to those found within cells. In addition, when quinolones were used as monotherapy in experimental animals and humans infected with *Brucella*, the rates of relapse were unacceptably high. Therefore, quinolones should always be used in combination with other drugs, such as doxycycline or rifampicin.

Trimethoprim/sulfamethoxazole (TMP/SMZ, co-trimoxazole). TMP/SMZ in a fixed ratio of 1:5 (80 mg TMP/400 mg SMZ) is more active in vitro against *Brucella* species than either drug alone. Although initial studies with TMP/ SMZ reported good results, prospective, controlled, comparative trials demonstrated that the drug was associated with an unacceptably high rate of relapse. Consequently, TMP/SMZ should always be used in combination with another agent, such as doxycycline, rifampicin or streptomycin.

5.4 Treatment of complications of brucellosis

5.4.1 Spondylitis

Osteo-articular complications of brucellosis are common, occurring in up to 40% of cases in some series of patients. Some manifestations, such as sacroiliitis, do not appear to require special treatment. In contrast, spondylitis and osteomyelitis with related complications, such as para-vertebral and epidural abscesses, may require prolonged therapy, such as the continuation of doxycycline for eight weeks or more. Surgical drainage is rarely necessary.

5.4.2 Neurobrucellosis

The treatment of central nervous system complications of brucellosis poses a special problem because of the need to achieve high concentrations of drugs in the CSF. Since tetracyclines and aminoglycosides do not penetrate the blood/brain barrier well, it is recommended that drugs which achieve this, such as rifampicin or co-trimoxazole, be added to the standard regimen of doxycycline plus streptomycin. The optimal duration of treatment for neurobrucellosis has not been determined, however, most authorities recommend a minimum of six to eight weeks, and possibly longer, depending on the clinical response.

5.4.3 *Brucella* endocarditis

Although death from brucellosis occurs in less than 1% of cases, the complication most frequently leading to a fatal outcome is infective endocarditis. The treatment of *Brucella* endocarditis poses special problems because of the need to achieve bactericidal concentrations of drugs within the valvular vegetations. In addition, delays in making the diagnosis often result in progressive valve damage. For these reasons, both antimicrobial chemotherapy and surgical replacement of the damaged valve are often necessary. The combination of doxycycline plus an aminoglycoside results in rapid killing of the bacteria, and rifampicin or co-trimoxazole are used for their ability to penetrate cell membranes. Prolonged therapy is recommended (at least eight weeks), and therapy should be continued for several weeks after surgery when valve replacement is necessary.

5.5 Treatment of brucellosis during pregnancy

If promptly diagnosed, antimicrobial therapy of pregnant women with brucellosis can be life-saving for the fetus. Pregnant women and nursing mothers pose special problems with regard to the selection of appropriate drugs. All drugs cross the placenta in varying degrees, thus exposing the fetus to potential adverse drug effects. Tetracyclines are contraindicated in pregnancy owing to the potential for permanent staining of fetal dentition, and the susceptibility of pregnant women to drug-induced fatty necrosis of the liver and pancreatitis. The teratogenic potential of many drugs, such as the fluoroquinolones, rifampicin, and co-trimoxazole, are simply unknown. Fetal toxicity has been reported in pregnant women treated with streptomycin; however, there are no reports of toxicity with gentamicin. Consequently, the optimal therapy for brucellosis during pregnancy has not been determined with certainty. Co-trimoxazole has

been used in individual cases with reported success. Another alternative is rifampicin therapy for at least 45 days depending on the clinical outcome.

5.6 Treatment of brucellosis in children less than eight years of age

The optimal treatment for brucellosis in neonates and children less than eight years of age has not been definitively determined. Tetracyclines are contraindicated because of the potential for permanent staining of deciduous teeth and inhibition of bone growth. Doxycycline binds less to calcium than other tetracyclines, and may pose less of a risk, however, there are no studies to confirm this with certainty. Consequently, aminoglycosides, co-trimoxazole, and rifampicin are the drugs generally recommended. Co-trimoxazole and rifampicin are not recommended by the manufacturers for use in young children, and the rates of relapse are high when either agent is used alone. Satisfactory results have been reported with TMP/SMZ (8/40 mg/ kg/day twice daily orally) administered for six weeks plus streptomycin (30 mg/kg/day once daily intramuscularly) administered for three weeks or gentamicin (5 mg/kg/day once daily intravenously or intramuscularly) administered for 7 to 10 days. Alternatives include TMP/SMZ plus rifampicin (15 mg/kg/day orally) each administered for six weeks, or rifampicin plus an aminoglycoside. Until additional experience is obtained with these regimens, it is not possible to define the therapy of choice.

5.7 Post-exposure prophylaxis

With increasing use of live *Brucella* vaccines to immunize cattle (*B. abortus* strain 19 and RB 51) and sheep and goats (*B. melitensis* strain Rev 1), the problem of accidental self-inoculation by veterinarians is widespread. The majority of vaccine needle-stick injuries cause puncture wounds, but usually little vaccine is injected. However, a potential risk of infection remains and it is advisable to supplement local wound care and tetanus toxoid (when indicated) with a six-week course of doxycycline. It should be noted that *B. abortus* RB 51 is resistant to rifampicin. In contrast, splashing the eyes (conjunctival inoculation) with live Brucella vaccines is a very effective method for transmitting brucellosis. Consequently, for vaccine accidents involving the conjunctival route, local eye care and one or two drugs administered for the full six-week course is recommended. In addition, serum should be tested for antibodies to *Brucella* as soon after the accident as possible, to provide a baseline for follow-up in case symptoms occur.

5.8 Vaccines and immune system stimulants

There is no convincing evidence of benefit from administering *Brucella* vaccines or antigen preparations, nor for the use of immune system modulators, such as levamisole, in the treatment of human brucellosis. Caution should be exercised in the use of anti-inflammatory agents to deal with local complications. Where possible, specialist advice should be sought.

KEY POINTS ON TREATMENT OF BRUCELLOSIS IN HUMANS

- The essential element in the treatment of all forms of human brucellosis is the administration of effective antibiotics for an adequate length of time.
- Treatment of uncomplicated cases in adults and children eight years of age and older: doxycycline 100 mg twice a day for six weeks + streptomycin 1 g daily for two to three weeks.

OR

- Doxycycline 100 mg twice a day for six weeks + rifampicin 600 ~ 900 mg daily for six weeks.

6. Prevention of human brucellosis

As the ultimate source of human brucellosis is direct or indirect exposure to infected animals or their products, prevention must be based on elimination of such contact. The obvious way to do this – elimination of the disease from animals – is often beyond the financial and human resources of many developing countries. The technical and social difficulties involved in eradicating *B. melitensis* from small ruminants has even taxed the resources of some developed countries. In many situations there is little alternative but to attempt to minimize impact of the disease and to reduce the risk of infection by personal hygiene, adoption of safe working practices, protection of the environment and food hygiene. The lack of safe, effective, widely available vaccines approved for human use means that prophylaxis currently plays little part in the prevention of human disease.

In industrialized countries and others where animal husbandry is practised under settled conditions, the main sources of infection are:
1) occupational exposure;
2) ingestion of contaminated food products.

Under conditions of nomadic or migratory husbandry or on small traditional farms, the differentiation of sources of infection is far less clear-cut and all sections of the population may be exposed to infection by direct contact with animals or from contaminated food.

Although the practice of even elementary hygienic precautions can be difficult for populations living under primitive conditions, especially in arid or semiarid areas, the observance of some basic measures can considerably reduce the risk of brucellosis.

6.1 Occupational hygiene

The groups in which the occupational risk of infection is greatest include those whose work brings them in direct contact with infected animals or their products. These include farmers, stockmen, shepherds, goatherds, abattoir workers, butchers, dairymen, artificial inseminators, veterinarians and those involved in the processing of viscera, hides, wool and skins. Persons involved in the maintenance of buildings or equipment used for these purposes may also be at risk. An additional important category includes laboratory workers who may be exposed to contaminated specimens and to *Brucella* cultures, either during the course of diagnostic procedures or vaccine production, for example. The production and use of live vaccines also carries some risk.

6.2 Personal hygiene

All persons carrying out high-risk procedures, which includes contact with animals suffering from or suspected of having brucellosis, should wear adequate protective clothing. This includes an overall or coat, rubber or plastic apron, rubber gloves and boots and eye protection (face shield, goggles or respirator). The risk of infection is greatest when dealing with aborting animals or those undergoing parturition but hazardous activities also include contacts with infected animals in other circumstances like shearing, dipping, clinical examination, vaccination and treatment, and the disinfection and cleaning of contaminated premises.

The work clothes should be reserved for this purpose and retained on the premises. They should be disinfected after use either by heat treatment (boiling or steaming), by fumigation with formaldehyde or by soaking in a disinfectant solution of appropriate concentration (iodophor, phenolic soap, chloramine or hypochlorite). Particular attention should be given to the disinfection of footwear to ensure that infection is not transferred outside the premises or into the house or tent.

Ideally, operatives should have access to full washing or showering facilities. As a minimum, the hands should be rinsed in a 1% chloramine solution (or other approved disinfectant), washed in soap and water and then treated with an emollient cream. Any superficial injuries such as cuts or scratches should be treated with an antiseptic, e.g. tincture of iodine, and covered with a bandage or self-adhesive dressing.

Eye protection is particularly important as conjunctival contamination carries a high risk of infection. Should any infectious material enter the eye, it should be removed under clean or aseptic conditions away from the working area. The eye should be thoroughly rinsed with running water and chloramphenicol or tetracycline eye drops or ointment applied.

Respiratory contamination is also a high risk in heavily infected environments. Inhalation of dust or aerosols derived from dried excreta or tissues released at abortion, parturition or slaughter should be prevented by the use of suitable respirators. The filters, which must be capable of retaining bacteria, should be changed regularly and the equipment itself disinfected by chemical or moist heat treatment.

Ideally, staff should be kept under medical surveillance with periodic serological examinations. It is strongly recommended that new staff provide a baseline blood sample before starting work. Any that develop clinical disease should be treated promptly. Young people under 18 years of age and pregnant women should be excluded from high risk occupations.

6.3 Farm sanitation

Farm workers, and animal attendants in particular, should wear adequate protective clothing when contact with infected animals is probable or if the environment is likely to have been contaminated by excreta, abortions or parturition products from animals with brucellosis. This is particularly important when dealing with animals that are aborting or giving birth, when the shedding of *Brucella* organisms will reach maximum levels.

Aborted fetuses, placentae and contaminated litter should be collected in leak-proof containers and disposed of preferably by incineration. Deep burial in freshly slaked lime at sites away from water courses is an acceptable alternative. Any area in which an abortion or infected parturition has occurred should be washed down with an approved disinfectant (hypochlorite, iodophor or phenolic disinfectant at recommended working strength).

Farm implements used for handling contaminated material should be disinfected after use by immersion in a suitable disinfectant (iodophor, phenolic soap or dilute caustic soda).

Dung should be cleared daily and stored in a secluded area until rendered safe by natural decay (this will probably require about one year) or else burnt or soaked in disinfectant before disposal. Liquid manure can remain infected for long periods, especially at low temperatures. Destruction of *Brucella* organisms can be hastened by addition of calcium cyanamide or xylene but the material should still be stored for at least six months.

Vehicles entering or leaving infected premises should pass through shallow troughs of disinfectant, or over straw or plastic foam soaked in an approved disinfectant. Premises that have held *Brucella*-infected animals should not be re-stocked until at least four weeks have elapsed between cleaning and disinfection. Maintenance workers (e.g. builders, plumbers, electricians) should not be allowed on to premises that have not been decontaminated.

Buildings should be maintained in a condition which prevents ready access to vermin. Rodent control measures should be enforced and insect infestation kept to a minimum by the use of fly screens, light traps and insecticides.

6.4 Prevention of brucellosis under nomadic or migratory conditions

Even in some developed countries sheep and goat raising is often practised under semi-nomadic conditions. These do not readily lend themselves to the hygienic measures indicated for farms. The situation is even more difficult when animal husbandry is practised on a completely nomadic basis in arid or semi-arid conditions. Under these circumstances it is rarely possible to follow hygienic practices to the extent required to prevent infection. However, steps can be taken to reduce the impact of the disease by educating the population in the nature of the disease and its mode of transmission. In such societies, most of the adult population will already have been exposed to brucellosis and will presumably have some degree of immunity. The disease therefore makes its greatest impact on children who should be prevented from having contact with newborn animals or those that have recently aborted or given birth. Although cultural traditions can be difficult to change, the consumption of raw milk, blood or uncooked meat or offals should be discouraged.

Vaccination is often the only measure that can be applied to such populations. However, fully satisfactory vaccines are not currently available.

6.5 Hygienic precautions in meat processing establishments and rendering plants

Sheep, goats and cattle infected with *B. melitensis* or *B. abortus* and pigs infected with *B. suis* are particularly dangerous at the slaughter stage. During the bacteraemic phase of the disease, the bacteria are widespread in the tissues. The mammary glands, uteri and testes may be particularly heavily infected. Animals that have recently aborted or given birth may also have extensive external contamination.

Cattle infected with *B. melitensis*, especially if pregnant or in milk, may shed enormous numbers of bacteria on opening the uterus or udder and present a severe risk to abattoir workers. It is advisable to dry off such animals before slaughter or, if this is not practicable, to incinerate the whole carcass.

If animals are known to be infected with *Brucella*, they should be slaughtered at abattoirs designated for that purpose, where the staff have been specially trained and equipped to deal with the risk. The slaughtermen should wear full protective clothing including waterproof overalls or aprons, boots, respirators and goggles or face shields. Rubber gloves must be worn and chainmail guards should be used to protect against accidental cuts. Eating, drinking and smoking must be prohibited in the working area. Adequate facilities for disinfection of protective clothing, implements and for personal washing should be provided.

If specially designated abattoirs are not available, the slaughter of infected animals should take place at the end of the working day, after slaughter of healthy animals has been completed. Tissues that are likely to be heavily infected, such as udder and genitalia, should be destroyed.

Full cleaning and disinfection of the premises and the equipment must be performed at the end of each working day. Animal tissues and refuse for disposal should be retained in leak-proof containers such as plastic bags. It is recommended that such material be incinerated.

Entry to the premises should be restricted to employees. Young persons under the age of 18 and pregnant women should not be allowed access. If possible, staff should be recruited from individuals known to have serological evidence of previous exposure to *Brucella*.

The staff should be kept under medical surveillance and antibiotic therapy implemented for any who develop symptomatic brucellosis. All employees and especially women of childbearing age must be apprised of risks

associated with *Brucella* infection. Information regarding conditions affecting immune status (e.g. pregnancy, immunosuppressive drugs, neoplasms, etc.) should be provided to workers. Personnel should be encouraged to self-identify to the organization's medical authority/health care provider so they may receive appropriate medical counselling and guidance. Education in safe and hygienic working practices and containment practices should be ongoing and is especially important for new staff.

6.6 Safety measures in the laboratory: precautions required in handling materials that may contain pathogenic *Brucellae*

Brucellae fall into WHO Risk Group 3, i.e. pathogens that pose a high risk to the worker involved, but only a low risk to the community. Brucellosis is in fact one of the most easily acquired laboratory infections. The degree of risk varies, not only with the virulence of the organism, *B. melitensis* and *B. suis* being the most dangerous for humans, but also with the numbers of bacteria in the material being handled. Blood samples and biopsy material for either serological or bacteriological diagnosis will rarely contain *Brucellae* in sufficient numbers to present a significant risk to personnel handling them but should still be handled with care at Biosafety level 2. Normally these will be dealt with in general diagnostic sections along with samples that may contain other human pathogens (Fig. 8). However, after *Brucellae* have grown in culture, dangerous numbers of organisms are present and strict precautions are required. At the same time Biosafety level 3 facilities, practices and procedures are required. The same applies when handling birth products from animals. Clotted blood samples present little risk and milk samples only a slight risk. Membranes, fetal tissues and fluids may contain up to $>10^9$ *Brucella* cells per gram, and similar numbers may be encountered in handling cultures grown in the laboratory. The precautions described below apply to handling this dangerous material. For a fuller treatment of the subject, the *WHO Laboratory Biosafety Manual* (3rd ed.) should be consulted; it contains much valuable information on biosafety in the laboratory and a bibliography of the subject.

6.6.1 Physical requirements for a laboratory handling pathogenic *Brucellae*

When handling cultures and other potentially high-titered materials such as membranes, fetal tissues and fluids Biosafety level 3 is prudent. A separate room is required with only one entrance; a biohazard notice prohibiting the entry of unauthorised persons should be prominently displayed at the entrance. Ideally, the room should have a double-door entrance designed to provide an airlock. The ventilation should be arranged to maintain the pressure within the room at a slightly lower level than its surroundings. Air from the room should be discharged to the exterior, well away from air intakes and opening windows, otherwise it must be sterilized by filtration or heat treatment. The walls should be impermeable and all windows sealed to allow disinfestation and fumigation; it should be safeguarded against infestation with rodents or insects. The room must have a properly installed and tested Class II or III biological safety cabinet. The air exhaust from the cabinet should be so arranged as to avoid interference with the air balance in the room or within the cabinet when it is switched on. The room should have a sink, an autoclave and enough incubator space for all culture requirements. Hand washing facilities must be provided near the exit.

6.6.2 Biological safety cabinets

See the *WHO Laboratory Biosafety Manual*, 3rd edition. For appropriate and useful information on selection and use of biological safety cabinets.

6.6.3 General precautions

Since brucellosis has been documented as one of the most frequently acquired laboratory infections the importance of using appropriate biosafety practices and facilities cannot be over emphasized. Each laboratory

should have written procedures addressing use of equipment (especially equipment that may generate aerosols); disinfection of equipment and contaminated materials, handling and processing samples; spill containment and cleanup; and waste handling. These procedures should be clearly and concisely written, easily accessible and rigorously followed. As previously mentioned, Biosafety level 3 is appropriate for handling *Brucella* cultures or infected membranes, fetal tissues and fluids.

6.6.4 Measures for specific laboratory processes

In the production of antigens, strains of low virulence such as *B. abortus* strain 1119-3[1], strain 19[1,2] or strain 99[2] should be used; they have the additional advantage that they do not require added CO_2 for growth.

Centrifuges may cause dangerous aerosols, especially when tubes containing virulent bacteria break. Glass tubes should not be used for virulent materials, instead polycarbonate tubes with tightly fitting screw-capped lids are recommended. If virulent material has to be centrifuged, the containers should be loaded and unloaded inside the biohazard cabinet. The continuous-flow type of centrifuge should not be used for virulent strains. Electric homogenisers, stomachers, sonicators and similar appliances should be used inside the cabinet.

Except for grossly contaminated materials, direct culture is the preferred method for bacteriological diagnosis rather than the inoculation of laboratory animals. If the inoculation of potentially dangerous materials is required, only needles that lock to the barrel of the syringe should be used. Needles should not be re-capped before disposal. The operators should wear protective clothing and respirators.

6.6.5 Health and medical surveillance

All persons working with virulent *Brucellae* should be kept under close clinical and serological surveillance. In some countries prophylactic immunization is offered to those at special risk. However, the vaccines currently available are of uncertain efficacy and some may cause unacceptable reactions.

6.7 Prevention of foodborne brucellosis

For the general population which does not have direct contact with animals, the greatest potential source of brucellosis is through consumption of unpasteurized milk and dairy products. Meat may also be a significant source of infection, especially in cultures where the consumption of raw or undercooked meat products is favoured.

6.7.1 Milk and milk products

Milk from infected cattle, sheep, goats, buffalo, yaks, camels and reindeer can contain large number of *Brucella* organisms. Because quite large volumes may be consumed or concentrated into other products, such as cream or cheese, it presents a particularly serious hazard.

Soft cheeses prepared from fresh milk may concentrate large numbers of *Brucella* organisms. The preparation of such products from untreated milk should be strongly discouraged. If local customs make this difficult to achieve, the cheese should be stored for six months before being released for consumption. Hard cheeses which may undergo propionic as well as lactic fermentation are usually much less hazardous because of the acidification. Unpasteurized whey left over from cheese making could transmit infection if fed to animals. It may also contaminate containers used to transport other materials unless these are decontaminated before use.

[1] Available from USDA National Veterinary Services Laboratories, P.O.Box 844, Ames, Iowa 50010, USA.
[2] Available from Veterinary Laboratories Agency, Addlestone, Surrey KT15 3NB, England.

Rennet used in cheese making can also serve as a source of infection if prepared from the stomachs of *Brucella*-infected animals.

Butter, sour milk, sour cream and yoghurt also undergo acidification processes which will drastically reduce the *Brucella* content. However, the acidity has to fall below pH 3.5 for reliable killing of the bacteria.

Ice cream prepared from infected milk may be particularly hazardous, especially as milk from different sources may be blended to make the product. All milk and cream used for this purpose should be heat treated.

Boiling or high temperature pasteurization will kill *Brucella* in milk. Ideally all milk produced in areas in which brucellosis is present should be pasteurized. If pasteurization facilities are not available, the milk should be heated to a minimum temperature of 80~85 °C and the temperature held at that level for at least several minutes, or boiled. This should apply to all milk for human consumption, whether to be drunk without further processing or to be used for making other food products.

6.7.2 Meat

Muscle tissue is unlikely to contain more than low concentrations of *Brucella* organisms and their numbers are further reduced if the meat is stored correctly before consumption. Kidney, liver, spleen, udder and testes may contain much larger numbers. None of them present a serious hazard from brucellosis if thoroughly cooked. However, in some cultures, raw or undercooked meat may be eaten through choice. This practice and the consumption of fresh blood, either alone or mixed with milk, should be discouraged.

The handling and preparation of infected meat and offal without proper hygienic precautions may be also lead to the contamination of other foods.

Drying, salting and smoking are not reliable methods for killing *Brucella*. Similarly, the organisms survive well under refrigeration or deep freeze conditions. It is strongly recommended that all meat products are thoroughly cooked before consumption.

6.8 Vaccines*

Safe and effective vaccines for the prevention of human brucellosis are not generally available. However, vaccination has played a significant role in the prevention of the disease, in conjunction with other measures, in the former USSR and China. Two live attenuated vaccine strains have been employed extensively in heavily infected areas.

B. abortus strain 19-BA was used from 1952 onwards in the former USSR. The vaccine was administered as a dose of 1 x 10^9 cells by skin scarification (epicutaneous route). Protection was effective for up to one year but with maximum efficacy at five to six months after vaccination. Accordingly, vaccination was usually timed to anticipate the season of peak incidence of disease in animals. In general, the vaccine was well-tolerated in healthy adults when given by the epicutaneous route. Local reactions manifested as hyperaemia and induration occurred in 76% of those immunized, whereas general reactions characterized by headache, lethargy and mild pyrexia, occurred in 3 to 7% of vaccinates. The frequency of general reactions was much greater in those showing evidence of previous exposure to *Brucella*.

Epidemiological studies showed that the vaccine was effective in reducing morbidity in high-risk areas, with a 5 to 11-fold reduction in reported cases of acute brucellosis. However, the vaccine did induce hypersensitivity, especially with repeated doses and there were numerous contra-indications to vaccination.

In China, the live attenuated strain *B. abortus* 104M has been used. This was administered as a dose of (7–10)

* See also *Manual of diagnostic tests and vaccines for terrestrial animals*, OIE, 5th ed.

x 10^9 viable cells given by the epicutaneous route. This strain is appreciably more virulent than *B. abortus* 19-BA and serious reactions may follow subcutaneous injection. The indications for use are similar to those for the 19-BA strain. Care must be taken to avoid vaccinating individuals who may have been sensitized by previous exposure to vaccine or natural infection.

These live vaccines are not currently available from sources whose production and quality control procedures would meet international standards. Their availability and use is now quite restricted. More emphasis in recent years has been on the development of non-living vaccines based on sub-cellular fractions. Two of these have received fairly extensive study.

A peptidoglycan fraction (PI) obtained as the phenol-insoluble residue of lipid extracted cells of *B. melitensis* M15 was developed in France. This was subsequently prepared from *B. abortus* strain 19 cells. It has been used in occupationally exposed groups, particularly laboratory workers. Two doses of 1 mg each are given subcutaneously, separated by a two-week interval. The vaccine is non-toxic and rarely elicits generalized reactions. It is very weakly allergenic and does not cause severe sensitization. However, it is reported to result in enhanced lymphocyte proliferation responses to *Brucella* antigens which correlate with immunity. The protective response was reported to last up to two years. Although used in about 2000 individuals over nearly two decades, evidence of efficacy from controlled clinical trials is not available. The vaccine is not at present in production.

Another sub-cellular fraction, "*Brucella* chemical vaccine" (BCV), was developed in Russia. It is extracted from cell wall preparations of *B. abortus* strain 19-BA with 0.1N acetic acid and comprises a protein-polysaccharide complex. The vaccine is given in doses of 1 mg by intramuscular injection and stimulates only mild local and general reactions. It does not produce severe hypersensitivity responses even in previously exposed individuals. Protective immunity is comparable with that given by the live 19-BA strain. Studies involving the use of 75 000 doses in Kazakhstan indicated an efficacy of 79.6% for BCV and 76.6% for the live vaccine. However, BCV was at least 2~4 fold less likely to induce cutaneous hypersensitivity than the live vaccine. Repeated doses could be given after one year without risk of serious hypersensitivity reactions. This vaccine would appear to merit further evaluation under a wider range of conditions.

Other vaccines are currently under development, including live attenuated strains with defined attenuating mutations and LPS-protein conjugate vaccines.

6.9 Public health aspects

From a public health point of view, the main sources of brucellosis are either food-related or are dependent on contact with infected animals either in an occupational or recreational contact. Person-to-person transmission is not a significant problem except through blood or organ transfer which should be subject to proper control. Airborne or contact infection through environmental contamination may be a significant problem when infected animals pass through densely occupied areas, e.g. on the way to market. Appropriate measures should be taken to address these problems. A key means of achieving this is through education of the population, and especially those directly involved in the animal and food industries.

All measures should be integrated into adequately designed and effectively implemented control programmes. Close collaboration between public health and veterinary services as well as other relevant agencies is fundamental in order to meet the targets.

6.9.1 Public health education

Food safety is one of the principal pillars on which protection of human health resides. Humans are infected by *Brucella* mainly through inappropriately prepared and/or preserved food of animal origin.

There is no lack of scientific knowledge on the systems, technologies and procedures with which to implement safe food preparation and consumption. Conversely, there is a huge gap in knowledge among the population, especially in developing countries, on the significance of safe handling, cooking and preserving food. Furthermore, food processing plant owners are often uninterested in, or even fail to apply correctly, the known rules of food safety (see Annex 1).

Foodborne diseases, including brucellosis, cause considerable morbidity in populations in many parts of the world, having a major impact principally on young children and the elderly.

Other than human suffering, foodborne diseases cause substantial economic losses. These include loss of income and manpower, medical care costs, loss of food due to inadequacy of processing or spoilage. Therefore, public health education should be included among the essential activities to be performed within the framework of brucellosis control programmes or even as an independent activity. Health education is a difficult and extremely complex task. It cannot be regarded as effective if specific considerations referring to the community are not taken into account. These include: culture, beliefs, traditions, educational level, social status, occupation, age, etc. Hence, health education programmes should be aimed at targeted social groups. These should include physicians, veterinarians and farmers who may not be fully aware of the problem. They should be directed not only at specific measures but should also emphasize the responsibility of individuals for safeguarding and improving their own health and that of the community. The key objectives are to enable individuals to define their own problems and needs; to understand what can be done to deal with these problems using their own resources and external support and to decide on appropriate action. This is best achieved in the context of a detailed knowledge of the social and environmental background.

Elements of health education and methodology are referred to in Annex 2.

6.9.2 Community participation

Health programmes are unlikely to succeed if community participation is not an integral part of the structure and execution of these programmes at local level. Laws, regulations and veterinary policy measures alone will not bring the desired results. The whole community needs to be involved through health education in schools, in the workplace and in the population at large.

Firstly, the higher the level of self-reliance and social awareness, the more individuals and families will accept responsibility for protecting their animals and themselves from disease hazards transmitted directly, through food of animal origin, or through environmental vectors or fomites. The relevant community education programmes should concentrate on what people can do for themselves to improve their own health situation.

Secondly, community members should be involved in planning the programmes that will affect them personally. Local residents know local social structures, local situations, local resources and local needs.

Thirdly, community members should be fully involved as participants in the implementation of health programmes in their communities. They have the important advantages of speaking the local dialect, of knowing how to reach people and animals and of enjoying social acceptance. Annex 3 refers to community groups to be identified for participation in health education campaigns.

There is no single model for promoting community participation. The degree of community involvement in zoonoses control programmes will vary from situation to situation, and is often strongly influenced by social, cultural, political and economic factors. Only guiding principles can be provided which might be applicable in different settings, provided that the will exists to begin and sustain the efforts. The general public, especially communities in endemic areas, has to be made aware of the danger to health and of the economic importance of zoonoses and foodborne diseases. As far as possible, full use should be made of the mass

media. All available means of informing each community should be used but an effective method is discussion in small groups. In such discussions, the health worker (educator) suggests some kind of concrete action, for example, formation of working committees soon after the discussions. Such committees have proved to be extremely useful in the initial early phases of several control programmes.

6.9.3 Training of health workers and school teachers on public health education

As far as possible, the health educators should be drawn from the community in which they will be working. Everyone involved directly or indirectly in a control programme against zoonotic and foodborne diseases must carry out public health education. It is, therefore, essential that this subject have an important place in staff training. Such training should be planned and preferably imparted by a specialist who should also advise on the selection of appropriate educational methods, the preparation of educational material suited to local conditions, and finally to the various phases of the programme.

Most of the health educators selected will already be professionally active and frequently good field experts. Therefore, on training courses academic lessons should be cut drastically and other teaching techniques preferably used that secure more active participation. Problem solving, case studies, working in small groups and role playing are examples of such techniques.

The school represents the most important learning situation for a large and significant group of the population. Effective instruction of children will have an influence not only on their own lives but also on the next generation. Children are influenced primarily via two channels, parents and teachers. They need to convey the need to develop a healthy lifestyle and the acquisition of healthy habits. It should be remembered that many school age children and young people in endemic areas do not attend school on a regular basis. Therefore, health education needs to extend beyond the school environment to reach educationally deprived groups which often include those at high risk.

The workplace is another important location for health education. Workers in the food industry and the managers and owners of food preparation facilities should be instructed in the potential causes of foodborne disease and the means of avoiding them.

For further information on strategies which can be adopted in education programmes see Annex 3.

KEY POINTS ON PREVENTION OF BRUCELLOSIS IN HUMANS

- The prevention of human brucellosis is based on occupational hygiene and food hygiene.
- Vaccination is not generally recommended.
- All dairy products should be prepared from heat-treated milk.
- Consumption of raw milk or products made from raw milk should be avoided.
- Meat should be adequately cooked.
- Special precautions should be taken by laboratory workers.
- Physicians and health workers should be aware of the possibility of brucellosis.
- Public health education should emphasize food hygiene and occupational hygiene.

7. Prevention, control and eradication of animal brucellosis

The justifications for prevention of the introduction of brucellosis into populations of animals are the same as those for the control of the disease in populations which are already infected: economic benefits and the protection of public health.

Brucellosis is a zoonosis with a strong correlation between animal and human diseases. While public health measures such as pasteurisation and education have varying degrees of success, it remains primarily a veterinary responsibility to control brucellosis, including application of principles of epidemiology and animal husbandry. Intersectoral collaborative strategies for control and prevention of brucellosis are reported in Annex 7*.

7.1 Prevention

It is nearly always more economical and practical to prevent diseases than to attempt to control or eliminate them. For brucellosis, the measures of prevention include:

- Careful selection of replacement animals. These, whether purchased or produced from existing stock, should originate from *Brucella*-free herds or flocks. Pre-purchase tests are necessary unless the replacements are from populations in geographically circumscribed areas that are known to be free of the disease.
- Isolation of purchased replacements for at least 30 days. In addition a serological test prior to commingling is necessary.
- Prevention of contacts and commingling with herds of flocks of unknown status or those with brucellosis.
- If possible, laboratory assistance should be utilized to diagnose causation of abortions, premature births, or other clinical signs. Suspect animals should be isolated until a diagnosis can be made.
- Herds and flocks should be included in surveillance measures such as periodic milk ring tests in cattle (at least four times per year), and testing of slaughtered animals with simple screening serological procedures such as the RBT.
- Proper disposal (burial or burning) of placentas and non-viable fetuses. Disinfection of contaminated areas should be performed thoroughly.
- Cooperation with public health authorities to investigate human cases. Animal brucellosis, especially when caused by *B. melitensis*, can often be identified through investigations of cases in humans.

7.2 Control

The aim of an animal control programme is to reduce the impact of a disease on human health and the economic consequences. The elimination of the disease from the population is not the objective of a control programme, and it is implicit that some “acceptable level” of infection will remain in the population. Control programmes have an indefinite duration and will need to be maintained even after the “acceptable level” of infection has been reached, so that the disease does not re-emerge. In many countries, methods for the control of brucellosis are backed by governmental regulation/legislation. In others, no authorities exist. Therefore, the procedures for management of infected herds and flocks may vary widely. Nevertheless, certain principles apply, namely: 1) the reduction of exposure to *Brucella* spp. and 2) the increase of the resistance to infection of animals in the populations. These procedures may be further classified under the general categories of test and isolation/slaughter, hygiene, control of animal movement, vaccination.

* For more information on the sanitary standards for international trade of animals and their products, see *Terrestrial animal health code*, OIE, 14th ed.

7.2.1 Test and isolation/slaughter

There are no pathognomonic signs of brucellosis in animals at individual level; the occurrence of abortion storms in naive herds/flocks is usually a strong indicator of infection. Therefore, serological (and sometimes allergic) tests are the usual method of identifying possible infected animals. Bacteriological procedures are useful for confirming test results and for epidemiological studies.

The decision about slaughter of test-positive animals is made after regulatory, economic and prevalence factors are considered. In most cases, test and slaughter of positive animals is only successful in reducing the incidence if the herd or flock prevalence is very low (e.g. 2%). Retention of positive animals is less hazardous if the remaining animals have been vaccinated but should only be considered as a last resort. The isolation of test-positive animals is essential, especially during and after parturition.

The immediate slaughter of test-positive animals is expensive and requires animal owner cooperation. Compensation is usually necessary. Furthermore, the application of test and slaughter policies is unlikely to be successful with brucellosis of sheep and goats where the diagnostic tests are less reliable than in cattle. Test and slaughter is also unlikely to be successful in cattle if the remainder of the herd is unvaccinated, especially in large populations. Repeated herd or flock tests are necessary to further reduce the incidence of brucellosis and to confirm elimination.

7.2.2 Hygiene

The goal in the application of hygiene methods to the control of brucellosis is reduction of exposure of susceptible animals to those that are infected, or to their discharges and tissues. This is a classical procedure in disease control. Factors such as the methods of animal husbandry (e.g. commingling of herds or flocks), patterns of commerce, prevalence of clinical signs, type of facilities, and degree of dedication of the owners of animals, will also determine success. Owners are often poorly informed about disease transmission and recommendations, such as separation of parturient animals, can be difficult or impossible to implement.

Antibiotic treatment of known infected animals, or of those which are potentially exposed to them, has not been commonly used and it should be ruled out as an option in the control of brucellosis. A limited number of studies have shown rapid reductions in the incidence of brucellosis when the herd of flock was treated but this procedure is considered to be restricted in practice. Treatment has been used in animals of special breeding value, but because of the uncertain outcome it is not generally recommended.

7.2.3 Control of animal movement

This may be regarded as an aspect of hygiene. However, it is essential in any programme to limit the spread of brucellosis. Animals should be individually identified by brand, tattoo or ear tag. Unauthorized sale or movement of animals from an infected area to other areas should be forbidden. Similarly, importations into clean areas must be restricted to animals that originate from brucellosis-free areas, that have a herd/flock history of freedom from the disease and that have given negative reactions to recently performed diagnostic tests.

In practice, it is much more difficult to control the movement of camels and small ruminants kept under nomadic or semi-nomadic conditions than that of beef or dairy cattle kept under intensive conditions. The owners of herds and flocks may be accustomed to seasonal migrations which may cross national boundaries.

7.2.4 Vaccination

There is general agreement that the most successful method for prevention and control of brucellosis in animals is through vaccination. While the ideal vaccine does not exist, the attenuated strains of *B. melitensis* strain Rev.1 for sheep and goats and *B. abortus* strain 19 have proven to be superior to all others. The non-agglutinogenic *B. abortus* strain RB51 has been used in the USA and some Latin American countries, with

encouraging results. The source and quality of the vaccines are critical. The dosages and methods of administration, especially with Rev.1, vary and these can affect the results. Consequently, whole herd or flock vaccination can only be recommended when all other control measures have failed. When applied, the vaccinated animals must be identified by indelible marking and continually monitored for abortions resulting from the vaccine. Positive serological reactors and secretors must be removed from the herd on detection.

It is often recommended that vaccination with strains 19 and Rev.1 should be limited to sexually immature female animals. This is to minimize stimulation of postvaccinal antibodies which may confuse the interpretation of diagnostic tests and also to prevent possible abortions induced by the vaccines. However, field and laboratory studies have demonstrated that conjunctival administration of these vaccines makes the vaccination of the herd or flock a practical and effective procedure. Rapid herd immunity is developed and application costs are minimized. The lowered dose results in lower antibody titres and these recede rapidly. Several diagnostic tests have been developed which are useful in differentiating antibody classes. Of these, the complement fixation test and ELISA are currently the most widely used.

Vaccination of animals usually results in elimination of clinical disease and the reduction in numbers of organisms excreted by animals which become infected. Furthermore, animal owners are more likely to accept vaccination as a method of control since they are accustomed to this form of disease control. In many countries, vaccination is the only practical and economical means of control of animal brucellosis.

The worldwide trend towards more animal commerce and larger populations, along with limited resources, have made the control of brucellosis very difficult in many countries. Evaluation of the procedures used for the prevention and control of animal brucellosis should be performed. This should include surveillance of animals and humans and investigations of outbreaks. Procedures, including case definition and diagnostic tests, should be standardized and should be flexible enough to allow modification when new information becomes available.

7.3 Eradication

Eradication means the elimination of a pathogenic agent from a country or a zone (i.e. part of the territory of a country with a distinct animal health status). A highly organized effort is needed to reach eradication in either a territory and in a population. Eradication is conceptually very different from control: it is neither a casual nor an automatic consequence of a control programme, no matter how well planned and implemented the control programme is. It is based on sanitary measures and on an organization of activities completely different from those implemented for a control programme. Crucial factors for the success of an eradication programme are the implementation of an effective surveillance system with adequate laboratory support, and the understanding and sharing of objectives for eradication by the decision-makers, farmers, and all other stakeholders. To keep an unaffected population free from an infection, prevention measures must be implemented to segregate an infectious organism from a geographical area and its human and animal populations. Adequate knowledge of the local human and animal populations and of the territory is essential.

The strategies described above for prevention and control can be applied for eradication; however, they are not mutually exclusive, on the contrary they can be arranged in a cascade as shown in diagram 1.

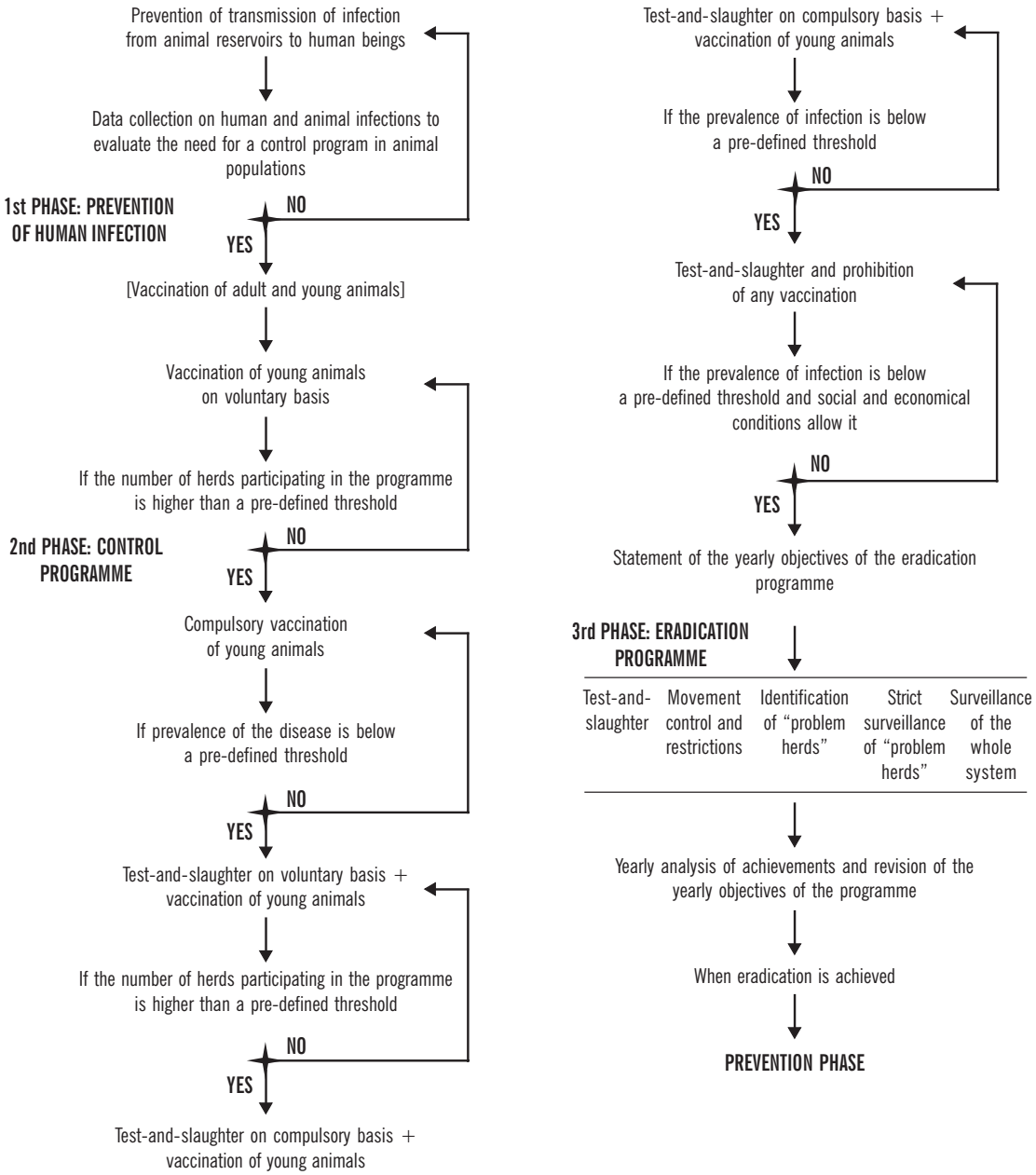
On a long-term basis, eradication programmes in general are more economically advantageous compared to control programmes. This advantage, however, cannot always be translated into practice. In fact, an eradication programme involves the mobilization of an amount of resources (financial and human) that may not be available or whose returns for the investment may require a time span longer than any decision-making authority can afford. Cost-benefit and cost-effectiveness analysis can be used to support decisions on control strategies. However, no in-depth analysis is possible in absence of epidemiological surveillance. There is also

little doubt that very often failures of control and eradication efforts are due to the absence of an adequate epidemiological surveillance system sustaining both technical and political decision-making.

KEY POINTS ON PREVENTION, CONTROL AND ERADICATION OF ANIMAL BRUCELLOSIS

- Animal brucellosis is best prevented by careful herd management and hygiene.
- Vaccination is useful for prevention and control of infection.
- *B. abortus* strains 19 and RB 51 are recommended for prevention of bovine brucellosis.
- *B. melitensis* Rev 1 is recommended for prevention of *B. melitensis* infection in sheep and goats.
- Vaccine efficacy may be limited in the face of heavy exposure.
- Control and prevention schemes require effective collaboration between all sections of the community.
- Control programmes must be properly planned, coordinated and resourced.
- Education and information programmes are essential to ensure cooperation at all levels in the community.
- Eradication can only be achieved by test-and slaughter combined with effective prevention measures and control of animal movements.

Diagram 1: Steps to achieve the eradication of brucellosis



Source: Mediterranean Zoonoses Control Programme. *The MZCP Report on the Third Workshop on Human and Animal Brucellosis Epidemiological Surveillance in the MZCP Countries*, Damascus, Syrian Arabic Republic, 4–5 May 1998 (http://www.mzcp-zoonoses.gr/pdfen/Brucellosis.pdf, accessed January 2007).

8. Surveillance

Surveillance consists of the systematic collection, collation, analysis, interpretation and prompt dissemination of data on specific diseases or syndromes to those who need to know, for relevant action to be taken. The main purpose of a surveillance system is to determine the need for immediate or longer-term actions in response to diseases and to provide information to optimize the use of the available resources through data analysis, determination of priorities, design of alternative actions, and determination of their likely costs and benefits. In relation to brucellosis, an effective surveillance system, based on the collaboration between the human and animal health services, is a prerequisite of any control or eradication programme. The surveillance system must be adapted to the adopted strategy for coping with the disease: prevention of human disease, and prevention, control or eradication of the infection in the animal population. In addition, other factors that will also determine the type of surveillance system include husbandry systems, marketing methods, and capacities of veterinary services.

The surveillance programme should be planned as an organisation made up of various components including institutions, facilities, activities and procedures with a common mission.

Collection and management of information is a costly activity; therefore, only the minimal set of information needed should be collected; and only the most suitable routinely performed activities should be used to collect information. A surveillance programme can be described in terms of inputs, processing and analysis, and outputs.

Inputs of a surveillance programme include passively or actively collected data. In the case of passive collection of information, data supply the system as a consequence of current activities. Main sources of passively collected data are: peripheral public health services, peripheral veterinary services, hospitals, public health laboratories, veterinary laboratories, border health services, border veterinary services. Other sources of data include clinics, physicians, veterinary practitioners, and universities. The laboratory is one of the main sources of data on zoonotic diseases. However, because of biases related to collection of samples in the population, laboratory data may not represent the sanitary situation of the population. Nevertheless, laboratories have a central role in generating information and, without them, a constant monitoring of animal health status for disease prevention and control cannot be carried out.

The active collection of data include those which are actively sought and gathered on the basis of a specific dedicated programme. Active collection is suitable for ad hoc surveys, to evaluate the performance of passive collection of data, to carry out pilot trials to evaluate if an emergent phenomenon deserves the implementation of a routine system of data collection. Ad hoc surveys are often the only possible way of collecting surveillance data when veterinary and health services do not have a strong infrastructure.

The following step in processing surveillance data is analysis, which aims at identifying and quantifying needs of health activities and evaluating their delivery. Indicators need to be identified to monitor progress.

The outputs of a surveillance system are, in general, technical reports on health conditions, resources available, their use and results obtained. Reports need to be adapted to the various recipient groups: peripheral collectors of data, intermediate and central technical people, decision makers, etc.

8.1 Surveillance in humans

The key to effective surveillance is the case definition, which includes the set of clinical and/or laboratory criteria that must be fulfilled in order to identify a person as a case. Individual reports of cases should be classified as suspected, probable or confirmed. Annex 8 includes the recommended standards for the surveillance of brucellosis in humans.

Monitoring of the number of cases reported by medical practitioners, clinics and hospitals can give an indication of the presence of the disease in a population. It is unlikely to give an accurate quantitative indication of the incidence as brucellosis is generally under-reported. Mild cases are particularly liable to be misdiagnosed or not reported.

Surveillance data can be actively collected by clinical and serological surveys on high-risk groups and on others, such as blood donors or pregnant women, who are accessible for examination. Surveys may also be conducted on patients admitted to hospital, military recruits and school children. Because of the absence of distinctive clinical signs, such studies have to depend on serological tests. These have to be interpreted with caution as exposure to cross-reacting organisms, including *Salmonella* 0:30, *Escherichia* coli O:157, *Yersinia enterocolitica* 0:9, may result in false positive reactions. Screening tests, such as the RBT, should be supported with more specific tests, such as IgG or IgA ELISAs, on at least a proportion of the samples.

Bacteriological screening of populations is not practical for surveillance purposes but cultures isolated from human patients should be identified to biovar level to enable possible tracking of sources and relation to outbreaks in animals.
The intradermal skin test has been widely used for epidemiological studies in some countries. This is even more difficult to interpret than serological tests and at best only gives an indication of past exposure. The specificity of the procedure is likely to be highly variable if inadequately standardized, and if unpurified *Brucella* preparations are used. These may also induce antibodies that interfere with subsequent serological tests. However, where other methods cannot be implemented e.g. through lack of laboratory facilities, intradermal testing with appropriate antigens may give a useful indication of level of exposure within a population.

8.2 Surveillance in animals

As for human brucellosis, a crucial factor is the case definition. The lack of any specific clinical presentation in animals makes the use of laboratory tests indispensable to define animal brucellosis cases. While the unit of reference for public health surveillance is the human case or cases (i.e. outbreak), for animal surveillance, the unit of reference is usually the infected herd or flock rather than the individual animal.

An animal brucellosis surveillance system may use data from diagnostic laboratory findings, outbreak/case investigations and slaughterhouse or animal marketing tests, or specially commissioned local or national surveys. These data can be used to ascertain flock or herd prevalence of a given population or area, and in infected flocks or herds, the prevalence of the disease in the flock or herd and to determine the incidence. An important use of incidence data is the evaluation of efforts to achieve control or elimination.

The following are methods for active and passive collection of data on animal brucellosis. Table 4 reports several recommended survey procedures according to the animal species under investigation.

- area tests (i.e. census) – the systematic testing of all animal herds or flocks within a geographical area;
- selected herd tests – the testing of animal populations considered to be higher risk: e.g. herds adjacent or commingled with infected herds;
- epidemiological investigations – the tracing of sources of animals which have been added to or sold from infected herds. It also includes attempts to locate sources of human cases of brucellosis. These are sometimes the first evidence of brucellosis in animals, especially for *B. melitensis*;
- surveys – tests of randomly selected herds to determine initial prevalence in an area or to monitor disease occurrence;
- tests of animals at slaughter or markets – This is a primary surveillance method in organized programmes where origins of positive animals can be located with reasonable confidence. The effectiveness lessens as the incidence in the population decreases. This form of surveillance is useful to determine initial prevalence;

- bulk milk ring tests – These are widely used to determine the prevalence of brucellosis in dairy cattle herds and to locate possible additional infected herds. Bulk milk (composite) samples are tested at least three to four times annually and individual cows are tested in herds where the ring test is positive. The test is very sensitive and false positive tests are common, especially where cattle herds are small or when cows have been vaccinated with strain 19.
- abortion investigations – In some countries abortions must be reported to authorities who are responsible for disease control.

Monitoring the males will indicate the presence of infection in the herd and drastically reduces workload.

The effectiveness and usefulness of any form of surveillance will depend upon the reliability and cooperation of animal owners and the availability and capabilities of diagnostic services.

KEY POINTS ON SURVEILLANCE BRUCELLOSIS IN HUMANS AND IN ANIMALS

- Continued surveillance is essential to monitor the presence/absence of brucellosis and the efficacy of control programmes.
- The key to effective surveillance is the case definition, reporting, analysis of data and dissemination of information for action.
- The surveillance programme must be designed according to the adopted control strategy.
- Human cases may be the first indication of infection in the animal population.

Table 4 Survey procedures recommended for the assessment of brucellosis epidemiology

ANIMAL		SURVEY PROCEDURE
CATTLE		
	DAIRY HERDS	Milk ring tests to identify infected herds and establish the prevalence of infected herds in the various regions·Blood samples from positive herds to establish the prevalence of reactors in infected herds Culture of milk from positive herds to support the serological data and identify the causal *Brucella* species and biovar Culture of abortion material
	EEF CATTLE	Serological tests on blood samples from breeding females sent to abattoirs, followed by identification of any infected farms Blood samples from infected farms Culture of lymph nodes and abortion material Serological surveys on live animals on farms and at markets, shows, and fairs
SHEEP, GOATS		Serological tests on blood taken at abattoirs, followed by identification of any infected flocks Serological tests in flocks suspected of being infected Surveys using allergic tests on selected herds followed by blood testing of those found positive Culture of lymph nodes and abortion material and milk samples from positive herds

ANIMAL	SURVEY PROCEDURE
PIGS	Serological tests on blood taken at abattoirs, including non-breeding females and castrated males, followed by identification of the source of any infected animals Blood tests in infected herds Culture of lymph nodes collected at abattoirs Culture of abortion material
FERAL OR WILD-LIFE SPECIES in contact with domestic animals	
	Capture or shooting and serological examination, supported by isolation and identification of the organisms

Source: Joint FAO/WHO Expert Committee on Brucellosis. Sixth Report, Technical Report Series, No. 740, World Health Organization, Geneva, 1986.

9. Intersectoral collaboration

In the preceding sections, the zoonotic nature of brucellosis has been emphasized. This implies that the disease in man can only be prevented effectively by elimination of the animal reservoir. This necessitates a close interaction between the medical authorities concerned with public health authorities on the one hand and the veterinary authorities on the other. This collaboration is only the first step in establishing an effective control programme. For a successful outcome, all sections of the community need to be involved in the process and to lend their support. This extends from the individual citizens who need to be aware of the measures required to protect and improve their own health, through local to national political leaders who will need to find and commit the resources required to implement the programme. Within this structure, the provision of specialist expertise is the responsibility of the medical and veterinary authorities. They will be responsible for diagnosis, treatment and surveillance and for executing control and preventive measures. They will also need to provide the necessary information to those concerned in occupational and community education programmes. The importance of this interaction and collaboration between sectors cannot be over-emphasized. Specific examples of approaches which may be adopted are given in Annex 3.

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Annex 1

Five keys to safer food[1]

- Keep clean
  - Wash your hands before handling food and often during food preparation
  - Wash your hands after going to the toilet
  - Wash and sanitize all surfaces and equipment used for food preparation
  - Protect kitchen areas and food from insects, pests and other animals
- Separate raw and cooked
  - Separate raw meat, poultry and seafood from other foods
  - Use separate equipment and utensils such as knives and cutting boards for handling raw foods
  - Store food in containers to avoid contact between raw and prepared foods
- Cook thoroughly
  - Cook food thoroughly, especially meat, poultry, eggs and seafood
  - Bring foods like soups and stews to boiling to make sure that they have reached 70°C. For meat and poultry, make sure that juices are clear, not pink. Ideally, use a thermometer
  - Reheat cooked food thoroughly
- Keep food at safe temperatures
  - Do not leave cooked food at room temperature for more than 2 hours
  - Refrigerate promptly all cooked and perishable food (preferably below 5°C)
  - Keep cooked food piping hot (more than 60°C) prior to serving
  - Do not store food too long even in the refrigerator
  - Do not thaw frozen food at room temperature
- Use safe water and raw materials
  - Use safe water or treat it to make it safe
  - Select fresh and wholesome foods
  - Choose foods processed for safety, such as pasteurized milk
  - Wash fruits and vegetables, especially if eaten raw
  - Do not use food beyond its expiry date

[1] *Prevention of foodborne disease: Five keys to safer food*. Geneva, Switzerland, World Health Organization, Department of Food Safety, Zoonoses and Foodborne Diseases (http://www.who.int/foodsafety/consumer/5keys/en/index.html, accessed January 2007)

Annex 2

Methodology for health education of the public

The content and form of the educational material and aids as well as means of communication have to be adapted carefully to the target populations and also to the health action about which information is to be prepared.

Most of the target populations in different parts of the world where brucellosis is endemic are illiterate or only partly literate. Written words, pamphlets and newspapers are therefore of little value in such situations. Fortunately, radio and television are spreading fast in various countries and portable radio sets are being carried even by nomads. These mass media are available for health education provided that the material is presented in a useful and interesting form.

Small discussion groups and lectures are extremely useful means of communication and could be followed immediately by such actions as formation of action committees or even by collection of diagnostic samples or immunization. Several audiovisual aids are available which could be used in conjunction with lectures or group discussion with great advantage. Others, such as posters and wall pictures, can be used on work premises to remind workers of various dangers or of precautions they have to take in handling potentially infected animals or products. It is important to enlist the aid of community leaders in the education campaign.

The content of the educational material including lectures, etc. has to be selected and prepared to suit the action to be supported and the beliefs and perceptions of the target populations. The topics treated in the rest of this section could each be the subject of educational communication, but each has to be broken down into very simple and easily comprehensible parts. Consideration of popular beliefs, however absurd they may appear, is important for the educator. If they are ignored, then resistance and lack of cooperation may follow. The correction of wrong beliefs should be done appropriately but in a gentle manner. The economic benefits expected to result from the control of brucellosis should be brought out fully in the educational material.

Many animal owners and patients do not like to have blood samples drawn for diagnostic or other purposes. Some object also to needle pricks for bleeding or injections. There are other real or imaginary fears of pain or injury resulting from health action. Many farmers do not cooperate for fear of immediate or future expense and others withhold cooperation simply due to ignorance. All these factors have to be taken into full consideration by the health educator in preparing his education aids and materials.

(Source: S.S. Elberg. A guide to the diagnosis, treatment and prevention of human brucellosis (unpublished document VPH/ 81.31. Rev.1). World Health Organization, Geneva, Switzerland, 1981)

Annex 3

Public health education groups for community participation

The following groups, which are to be found in most communities, are important:

1. Local healthy and veterinary services. The personnel of these services are not only participants in community programmes, but serve at the same time as educators and promoters.
2. Local health committees and community health workers. This group is most important for community motivation and education in the course of their work.
3. Local religious bodies. They guide both the attitudes and the actions of the people in many countries. Their advocacy of health programmes is essential. They can often make such invaluable facilities as meeting halls, audiovisual equipment and communication networks available to community projects.
4. Local civic groups. Dedicated to community improvement, they bring together civic leaders and have resources in the form of personnel and funds that can be extremely helpful in community projects.
5. Local school and adult education groups. Located within the communities, they can reach entire families, have facilities and resources for group meetings, attract the respected educated people in their communities, and can play an invaluable part in health programmes.
6. Local practitioners of traditional medicine, birth attendants and midwifes. Often respected by large segments of their communities, they should be involved in health programmes and actively participate in them, whenever possible.
7. Local police or local military units. Often anxious to participate actively in community service, these groups must be informed of, and involved in all programmes within their communities.

Both in rural and urban areas, community groups are all-important in the planning and implementation of health programmes. They provide the resources needed for adapting plans to local conditions, carrying out tasks at little or not cost, and overcoming constraints. They must be informed in their approach and informative about their role in achieving the aims of the programme.

(Source: *Bacterial and Viral Zoonoses*. Technical Report Series 682, World Health Organization, Geneva, Switzerland, 1992)

Annex 4

Table A.1 Characters differentiating the nomen species and biovars of Brucella

Species	Biovarand biogroup	CO_2 requirement	H_2S production	Urease	Thionin	Basic fuchsin	A	M	R	Preferred natural host
					Growth in presence of l in 50 000 (20 mg/ml) Agglutination by monospecific serum[+]					
B. melitensis	1	–	–	+	+	+	–	+	–	Sheep, goat
	2	–	–	+	+	+	+	–	–	Sheep, goat
	3	–	–	+	+	+	+	+	–	Sheep, goat
B. abortus*	1	(+)	+	+	–	+	+	–	–	Cattle
	2	(+)	+	+	–	–	+	–	–	Cattle
	3	(+)	+	+	+‡	+	+	–	–	Cattle
	4	(+)	+	+	–	(+)	–	+	–	Cattle
	5	–	–	+	+	+	–	+	–	Cattle
	6	–	–	+	+‡	+	+	–	–	Cattle
	9	–	+	+	+	+	–	+	–	Cattle
B. suis	1	–	+	+	+	(–)	+	–	–	Pig
	2	–	–	+	+	–	+	–	–	Pig, hare
	3	–	–	+	+	+	+	–	–	Pig
	4	–	–	+	+	(–)	+	+	–	Reindeer
	5	–	–	+	+	–	–	+	–	Rodents
B. neotomae	–	–	+	+	–	–	+	–	–	Desert wood rat
B. canis	–	–	–	+	+	(–)	–	–	+	Dog
B. ovis	–	+	–	–	+	(–)	–	–	+	Sheep
'B. maris' #	1	+	–	+	+	+	+	+ or –	–	Seals
	2	–	–	+	+	+	+	+ or–	–	Cetaceans

+ Positive; (+) usually positive,
– Negative, (–) usually negative.
* Former B. *abortus* biovars 7 and 8 are no longer regarded as valid
† A: abortus; M: melitensis; R: rough
‡ B. *abortus*, biovar 3 grows in the presence of 1 in 25 000 thionin; biovar 6 does not.
# 'B. maris' includes several distinct types and each may be accorded nomen species status

Table A.2 Oxidative metabolism of species of *Brucella*

Species	Amino acids							
	L-alanine	L-asparagine	L-glutamine acid	L-arginine	DL-citulline	L-lysine	DL-ornithrine	
B. melitensis	+	+	+	–	–	–	–	
B. abortus	+	+	+	–	–	–	–	
B. suis	v*	v*	v*	+	+	v*	+	
B. neotomae	v	+	+	–	–	–	–	
B. canis	v	–	+	+	+	+	+	
B. ovis	v	+	+	–	–	–	–	
B. 'maris'	–	–	+	–	ND	–	–	
	Carbohydrates							
	L-arabinose	D-galactose	D-ribose	D-xylose	D-glucose	i-erythritol		
B. melitensis	–	–	–	–	+	+		
B. abortus	+	+	+	v	+	+		
B. suis	v*	v*	+	+	+	+		
B. neotomae	+	+	v	–	+	+		
B. canis	v	v	+	–	+	v		
B. ovis	–	–	–	–	–	–		
B. 'maris'	ND	v*	+	+	+	+		

\+ positive
– negative
v variation between strains
v* variation between biovars of some assistance in classification
ND no data

Table A.3 Lytic activity of phages for smooth (S) and rough (R) *Brucella* species

Phage group	*Phage strain*	*abortus*		*suis*		*melitensis*	*neotomae*		*canis*	*ovis*	*maris*	
		S	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**R**	**R**	**S**
1	Tb	L	NL	NL	NL	NL	NL	NL or PL	NL	NL	NL	NL
2	Fi 75/13	L	NL	PL	NL	NL	NL	L	NL	NL	NL	L
3	Wb	L	NL	L	NL	V	NL	L	NL	NL	NL	L
4	BK_2	L	NL	L	NL	L or PL	NL	L	NL	NL	NL	L
5	R/C	NL	L	NL	NL	NL	NL	NL	NL	L	L	NL
6	Iz_1	L	NL	bg 1, 4: L bg 2,3,5: PL	bg 1,4: V bg 2,3,5: NL	L or PL	V	L	PL	NL	NL	L
7	Np	L	NL	NL	NL	NL	NL	L	NL	NL	NL	ND

L confluent lysis
PL partial lysis, single plaques or growth inhibition
NL no lysis
V variable, some strains lysed
RTD routine test dilution
ND no data
bg biogroup

Annex 5

Bacteriological examination for presence of *Brucella*

• Procedure

Blood (5～10 ml) or other specimen:

a) Liquid medium (50～100 ml per bottle, and containing 1%～2% sodium citrate when blood is to be studied)
OR
Liquid – solid combined medium (biphasic or Castañeda culture) Incubation at 37 °C until growth appears

b) Subculture from I on to agar medium
Incubate as above allowing 4～5 days for colony formation if necessary

c) Colonies examined for "Smooth-Rough" quality and by slide agglutination test in anti Brucella serum

d) Confirm the presumptive evidence of *Brucella* isolation by standard taxonomic tests on subculture

• Culture media

Both solid and liquid media may be used. Both may be prepared from similar ingredients. A good quality peptone is essential for the basal medium. Trypticase Soy Broth (BBL®), Bacto-tryptone® (Difco Laboratories GmbH), Tryptic soy (Gibco), Tryptone Soya Broth (Oxoid Ltd) and Trypticase soy (bioMérieux) are all suitable. To prepare liquid medium, sterile equine or bovine serum (which must be free of Brucella antibodies) and dextrose are added to the autoclaved peptone solution cooled to 56 °C, to give final concentrations of 5% v/v and 1% w/v, respectively. For solid medium, agar to 1.5% final concentration is added to the peptone solution before autoclaving.

Selective media: modified Brodie and Sinton liquid medium

To prepare this, antibiotics are added to the serum dextrose broth to give final concentrations as follows:

bacitracin:	25 u/ml
cycloheximide:	100 u/ml
polymyxin B sulphate:	6 u/ml
nalidixic acid:	5 μ/ml
vancomycin:	20 μ/ml
amphotericin B:	1 u/ml
D-cycloserine:	100 u/ml

Selective media: Farrell's medium

This solid selective medium is prepared from serum dextrose agar by addition of antibiotics to the molten medium cooled to 56℃ , to give the following concentrations:

bacitracin:	25 u/ml
cycloheximide:	100 μg /ml
polymyxin B sulfate:	5 u/ml
vancomycin:	20 μg/ml
nalidixic acid:	5 μg/ml
nystatin:	100 u/ml

• Biphasic medium

To prepare a biphasic medium of Castañeda type, either non-selective serum dextrose broth and serum dextrose agar can be used to form the liquid and solid phases. If a selective medium is required the antibiotics for the modified Brodie and Sinton medium are included in both phases. The bottles are

prepared as follows.

In a 125 ml flat-sided bottle place 12~14 ml melted agar medium (above, to which 2.5% agar has been added) to cover the longer side of the bottle, autoclave and leave to rest on the longer side until it hardens. When the medium has hardened, add aseptically to the bottle 15 ml sterile liquid medium with 1~2% sodium citrate. The bottle now has a solid medium on one side and a liquid medium on the bottom. The specimen is introduced into the liquid phase, the bottle incubated in the upright position. Every 24~48 hours the liquid is washed over the agar surface for a few minutes, the bottle returned to the upright position and further incubated. Eventually *Brucella* colonies will grow on the agar as well as in the liquid phase.

• Incubation

Incubation conditions must allow for the possibility of *B. abortus* that requires 10~20% additional CO_2 in the air. Cultures therefore must be made in duplicate at the beginning, one set incubated in air, the other set incubated in the presence of additional CO_2, until it is observed that the colonies have grown in air alone. At this point it is no longer necessary to provide additional CO_2 to the cultures. Most positive cultures become evident within one to two weeks. However, it is advisable not to discard cultures as negative until four to six weeks have elapsed.

Annex 6

Serological tests

Serum agglutination test

The test is performed in clear glass or plastic tubes of approximately 1~2 ml total volume by placing 0.8 ml of phenol saline (0.5% [w/v] phenol in 0.15 M sodium chloride) into the first tube and 0.5-ml volumes of phenol saline in the remaining tubes of a series of five or ten tubes. A volume of 0.2 ml serum is added to the first tube, mixed, and then 0.5 ml is transferred to the next tube. Further volumes of 0.5 ml are transferred to subsequent tubes to give a series of doubling dilutions. An equal volume of standard *B. abortus* agglutination suspension, diluted to working strength in phenol saline, is then added to each tube, and the tubes are incubated at 37 °C for 20 hours.

The tests are read against opacity standards prepared by diluting the working strength antigen 1 in 4, 2 in 4, and 3 in 4, to correspond to 25%, 50% and 75% agglutination. Phenol saline is used as the 100% control, and the undiluted working strength antigen as the 0% control. The results are scored as the degree of agglutination (1+ = 25%, 2+ = 50%, 3+ = 75%, 4+ = 100%) over the serum dilution. In each set of tests, a positive control serum calibrated against the International Standard for *B. abortus* antiserum (ISABS) must be included. This enables the results to be expressed in IUs and permits tests that have been performed in different laboratories to be compared. Titres have to be interpreted in the light of the patients' history and occupational background.

For cattle, titres equivalent to 50 IU or more for unvaccinated animals and 100 IU or more for vaccinates are regarded as indicative of infection.

Microagglutination methods using a stained antigen may be performed in microtitre plates instead of tubes.

2-mercapto-ethanol agglutination test

The 2-mercapto-ethanol test is carried out by diluting 0.1 ml of serum in 0.4 ml of 0.15 M sodium chloride (physiological saline) and adding 0.5 ml of 0.2 M 2-mercapto-ethanol in saline. The serum is then reduced by incubation at 37 °C for one hour, followed by serial doubling dilutions in saline. Volumes of 0.5 ml of standard SAT antigen diluted to working strength in saline (without phenol) are then added to each tube, and the test is subsequently performed as for the standard test. It is more closely correlated with active infection but no more sensitive than the standard SAT.

The test is useful in the examination of human sera as it may help to differentiate IgM agglutinins resulting from recent infection or exposure to cross-reacting antigens from the IgG agglutinins associated with active or long-standing infection.

Coombs antiglobulin agglutination test

The serum agglutination test is performed according to the recommended procedure. Following incubation at 37 °C, the cells are deposited by centrifugation, preferably in a refrigerated centrifuge at 4 °C. The supernatant liquid is then discarded and the cell deposit washed by resuspension in 0.15 M sodium chloride followed by centrifugation. This process is performed at least twice. The cell deposit is then finally re-suspended in 0.5 ml volumes of anti-human IgG serum diluted to working strength. The tubes are then reincubated at 37 ºC overnight. Agglutination is then scored as for the agglutination test. A human positive serum control and a saline negative control should be included in the series.

If IgA antibodies are to be detected, a broad specificity anti-human immunoglobulin reagent should be used.

This test has now been largely superseded by the ELISA.

Rose Bengal plate test (RBT)

Serum samples may be screened using the Rose Bengal plate agglutination test or card test Serum (0.03 ml) is mixed with an equal volume of antigen on a white tile or enamel plate to produce a zone approximately 2 cm in diameter. The mixture is agitated gently for four minutes at ambient temperature, and then observed for agglutination. Any visible reaction is considered to be positive. The test is very sensitive and positive samples should be checked by the CFT or by an IgG specific procedure such as ELISA. False-negative reactions occur especially in the early stages of acute infection. The RBT can be used in all animal species but positive results should be confirmed by a quantitative test. False positive results occur in vaccinated animals. False negative results are common in sheep, goats and pigs.

Complement fixation test (CFT)

Numerous variations of this exist but, whichever procedure is selected, the test must use an antigen that has been prepared from an approved smooth strain of B. abortus, such as strain 99 or 1119–3, and standardized against the second ISABS. Antigen for the CFT can be prepared by special procedures or antigen that has been prepared for the standard agglutination test can be used after diluting the stock suspension 1 in 200 in CFT buffer before standardization. The packed cell volume of the concentrated antigen suspension for CFT should approximate 2% before standardization against the second ISABS, as described below. The phenol concentration must not exceed 0.5%. The appearance of the antigen when diluted 1 in 10 in phenol saline must be that of a uniform, dense, white suspension with no visible aggregation or deposit after incubation at 37 °C for 18 hours. It must not produce anti-complementary effects at the working strength for the test. The antigen should not be frozen.

A convenient procedure is the microtitration method. All dilutions are made in a buffer prepared from a stock solution of sodium chloride (42.5 g), barbituric acid (2.875 g), sodium diethyl barbiturate (1.875 g), magnesium sulphate (1.018 g), and calcium chloride (1.147g) in 1 litre of distilled water and diluted by addition of four volumes of 0.04% gelatine solution before use (CFT buffer). The indicator system is a 3% suspension of fresh sheep red blood cells (SRBC) sensitized with an equal volume of rabbit antisheep RBC serum diluted to contain five times the minimum concentration required to produce 100% lysis of the SRBC in the presence of a 1/30 dilution of fresh guinea pig complement.

The latter is independently titrated to determine the minimum concentration required to produce 100% lysis of a sensitized SRBC suspension; this is defined as the unit of complement. The standard B. abortus agglutination test antigen should be diluted in a CFT buffer to a concentration that gives 50% fixation of complement (1.25 units) at a dilution of 1/200 of the second ISABS. The test sera are diluted in equal volumes of CFT buffer and inactivated by heating at 58 °C for 50 minutes.

Test procedure

a) Using standard 96-well U-bottom micro-titre plates, volumes of 25 µl of diluted test serum are placed in the wells of the first and second rows, and 25 µl unit volumes of CFT buffer are added to all wells except those of the first row.
b) Serial doubling dilutions are then made by transferring 25 µl volumes of serum from the second row onwards.
c) Volumes of 25 µl of antigen, diluted to working strength and 25 µl of complement at 1.25 units strength, are added to each well. Control wells containing diluent only, serum+complement+diluent, antigen+complement+ diluent, complement+diluent, are set up to contain 75 µl total volume in each case.

d) The plates are incubated at 37 °C for 30 minutes with agitation for the initial 10 minutes, or at 4 °C overnight.
e) Volumes of 25 µl of sensitized SRBC suspension are added to each well, and the plates are re-incubated at 37 °C for 30 minutes with occasional agitation for the first 10 minutes.
f) The results are read after the plates have been left to stand at 4 °C for two to three hours to allow unlysed cells to settle.

The degree of haemolysis is compared with standards corresponding to 0%, 25%, 50%, 75% and 100% lysis. Results should always be expressed in IUs, calculated in relation to those obtained in a parallel titration with a standard serum calibrated against the ISABS. In general, sera giving positive fixation at a titre equivalent to 20 ICFTU/ml or greater, are considered to be positive. However, for human sera, the history should be taken into account when interpreting the results of this test.

Indirect ELISA

Numerous variations of this indirect ELISA have been described. Commercial tests are available. Only those ELISAs that use *B. abortus* or *B. melitensis* smooth lipololysaccharide (LPS) are recommended. Tests are performed in 96-well, flat-bottomed, polystyrene microplates. The choice of microplate will have a slight effect on assay performance in terms of background activity observed. Low to medium protein-binding microplates give the lowest background activity using LPS antigen.

The antigen-coating buffer is 0.05 M carbonate-bicarbonate buffer, pH 9.6, composed of $NaHCO_3$ (2.93 g), Na_2CO_3 (1.59 g), and NaN_3 (0.2 g) in 1 litre distilled or deionised water. The conjugate and test sera diluent buffer is 0.01 M PBS, pH 7.2, + 0.05% (v/v) Tween 20 composed of Na_2HPO_4 (1.21 g), KH_2PO_4 in (0.20 g), NaCl (8.00 g), and KCl (0.20 g) in 1 litre distilled or deionised water + the addition of 0.50 ml Tween 20/litre. The wash buffer is 0.002 M PBS, pH 7.4 + 0.05% Tween 20.

The conjugate used should be a polyclonal antibody specific for both heavy and light chains of human IgG and conjugated to horseradish peroxidase. The substrate system is 4.4 mM H_2O_2 and 3.6 mM 2,2'-Azino-bis- (3 ethylbenzothiazoline-6-sulphonic acid) (ABTS) in 0.05 M phosphate/citrate buffer, pH 4.5, composed of 0.2 M Na_2HPO_4 (25.7 ml), 0.1 M citric acid (24.3 ml), and distilled/deionized water (50 ml); adjust the pH if necessary). The enzymatic reaction-stopping solution is 4% sodium dodecyl sulphate or 0.1 M NaN_3 in distilled/deionized water.

Test procedure

a) LPS antigen is diluted in coating buffer to a concentration determined by checker-board titration, usually approximately 1 ug/ml, and dispensed to all microwells in 100 µl volumes. The microplates are then incubated at 37 °C for two hours or at 4 °C overnight. As this is a solid-phase ELISA technique, the microplate wells require intervening washes between each assay step to remove unbound or unreacted reagents. From three to four wash cyclesusing the washing buffer, are sufficient. Prior to addition of the next reagent, the plates should be inverted and slapped onto a lint-free absorbent surface to discharge any residual contents.

b) Test sera and controls are diluted 1/200 in diluent buffer and applied to appropriate wells in 100 µl volumes. The plates arecovered or sealed and placed on an orbital plate shaker and incubated at 37 °C for one hour with continuous shaking. Wash the plate as above.

c) The enzyme conjugate is diluted in diluent buffer and applied to all wells in 100 µl volumes. The plates are covered or sealed and placed on an orbital plate shaker and incubated at 37 °C for one hour continuous shaking. The optimal dilution of conjugate should be such that when reacted with the strong positive control under standard conditions, it will result in an average absorbance value of between 1.0

and 1.4 absorbance units (see step f). A known positive Reference Serum should be used. Wash the plate as above.

d) Fresh substrate/chromogen solution is prepared by adding 60 ul of a 3% H_2O_2 stock solution to 12 ml of phosphate/citrate buffer containing 3.6 mM ABTS. The substrate/chromogen solution is applied to all wells in 100-µl volumes. The plates are transferred to an orbital plate shaker and incubated at 37 °C for precisely 15 minutes with continuous shaking. After 15 minutes incubation, the stopping solution is applied to all wells in 100-µl volumes and the plateis shaken briefly on the plate shaker to ensure thorough mixing. All wells now contain a total volume of 200 µl.

e) The colour development is read with a microplate photometer using a 405 or 414 nm interference filter.

f) The data may be expressed in a number of different ways, but it is recommended that test serum reactivity be expressed as per cent positivity of a standardized strong positive control serum. The strong positive control serum should be such that, when prediluted in negative serum, it exhibits an antibody activity that lies on the linear portion of the dose/response curve of the original high-titred serum, just below the plateau phase.

Annex 7

Intersectoral collaboration strategies for control and prevention of brucellosis

1. Strategies

The implementation of any control programme requires collaboration between many sectors of the community if it is to be successful. Development of prevention and control programmes should involve the participation of the community from the outset to provide a basis for efficient, effective and economical control. The elaboration of comprehensive national programmes should be based on the requirements of community programmes. Strategies in the development of such programmes are outlined below.

- To prevent the spread of infection amongst animals, and monitoring of brucellosis-free herds and areas.
- To carry out mass immunization to reduce the rate of infection in specified herds and areas.
- To control the spread of infection, implementation of non-specific measures in addition to specific measures, or as an alternative in areas where specific measures are not available.
- To eliminate infected animals by test and slaughter in order to develop brucellosis-free herds and areas.
- To inform and educate the general public, and train professional personnel (Annex 4, Table A.1).

These strategies are not mutually exclusive and the most effective programmes will combine all these elements.

2. Methods to be used in the field

Implementation of a programme would involve the application of various technologies applicable to field conditions. These will include:

- Analysis of the epidemiological situation (see details in Section 8, Table 4)
- Selection of herds/flocks and areas for action;
- Protection of low-prevalence and brucellosis-free areas (further details are given in the *OIE International Zoo-Sanitary Code*, 1997).

3. Planning, management and implementation of prevention and control measures

3.1 Steps initiated and sustained by the community

- Designation of a responsible person(s)
- Allocation of resources
- Annual assessment of the community programme(s)
- Identification of any further specific measures to be taken in collaboration with local government, and discussion of plan of action for epidemiological assessment and schemes for prevention and control
- Requesting intersectoral cooperation between different national services

3.2 Implement community – initiated schemes with the assistance of the government

- Elaboration of guidelines on simple decision-making processes
- Formulation of local plans of action, including:

– epidemiological surveys,
– education and information media on personal hygiene,
– immunization programmes,
– animal replacement schemes.

3.3 Development of various activities and services, in collaboration with peripheral governmental services

- Diagnostic services
- Vaccine provision in conjunction with use of cold chain
- Animal waste disposal or rendering
- Report on the progress of the programme to the central government

3.4 Comprehensive national programmes

- Establishment of an inter-ministerial committee
- Designation of a national programme directorate
- Preparation of guidelines for:
  – community activities
  – supporting services
  – comprehensive national plan
- Review and improvement of national rules and regulations
- Formulation of the countrywide programme of brucellosis control
- Institutional framework
- Mobilization of resources
- Programme implementation harmonizing the separate phases including the following aspects:
  – financial resources
  – geographical coverage
  – technologies
  – manpower
- Programme monitoring, periodic evaluation, and review.

All groups within the community, from ministerial to individual citizen level, must be kept informed of the status of the programme if it is to succeed.

3.5 International cooperation

In relation to the technical aspects of prevention and control of brucellosis, WHO, jointly with FAO and OIE, would wish to encourage and support programmes incorporating the above-mentioned international strategies on brucellosis control. Advice on human brucellosis may be sought from WHO. FAO and OIE can advise on the agricultural and international trade aspects of animal brucellosis.

Table A.4 Health education topics in the control of brucellosis

Group	Topic	Expected action
Livestock breeders	• Concept of brucellosis • Characteristics of the disease • Damage done to human health • Damage done to animal production· • Legislation backing the measures taken by the control agencies	• Collaboration with the measures of prevention and control of brucellosis carried out by public health and animal health services
Personnel that work in direct contact with animals (shepherds, milkers, farmer, abattoir workers, inseminators, veterinarians)	• Concept of brucellosis • Characteristics of the disease • Damage done to human health • Affecting species • Means of transmission to man • Preventive measures such as use of protective clothing, personal hygiene, environmental health	• Application of the recommended measures in order to prevent the disease
General population	• Concept of brucellosis and its importance as a zoonosis • Ways of transmission to man • Symptomatology in man • Methods of prevention, especially related to milk or fresh cheese consumption	• Positive attitude with respect to the care of their own health and acknowledgment of brucellosis as a human disease

Source: Joint FAO/WHO Expert Committee on Brucellosis. Sixth Report. Technical Report Series, No. 740, WHO, Geneva, 1986.

Annex 8

Recommended standards for surveillance, prevention and control of human brucellosis (A23)

General introduction

Brucellosis is a widespread zoonosis mainly transmitted from cattle, sheep, goats, pigs and camels through direct contact with blood, placenta, fetuses or uterine secretions, or through consumption of contaminated raw animal products (especially unpasteurized milk and soft cheese). In endemic areas, human brucellosis has serious public health consequences. Worldwide, *Brucella* melitensis is the most prevalent species causing human brucellosis, owing in part to difficulties in immunizing free-ranging goats and sheep. In countries where eradication in animals (through vaccination and/or elimination of infected animals) is not feasible, prevention of human infection is primarily based on raising awareness, food-safety measures, occupational hygiene and laboratory safety. In most countries, brucellosis is a notifiable disease.

Causal agent and main modes of transmission

- *Causal agent. Brucella abortus, biovars 1–6, 9; B. melitensis, biovars 1–3; B. suis, biovars 1,3 and 4; B. canis. B. suis biovar 2 and B. maris* infections have rarely been described. Infected animals (mainly cattle, sheep, goats, pigs and less commonly dogs and other animals) and their products are the reservoirs and sources of infection.

- *Main modes of transmission*. Ingestion, direct contact through breaks in the skin and airborne infection (laboratories and abattoirs), primarily affecting consumers of raw milk and derivatives, farmers, butchers, veterinarians and laboratory personnel. The incubation period is highly variable, usually 2–4 weeks, can be 1 week to 2 months or longer.

Clinical description and recommended case definition

Clinical *description*. Brucellosis may present with acute or insidious onset, with continued, intermittent or irregular fever of variable duration, profuse sweating, fatigue, anorexia, weight loss, headache, arthralgia and generalized aching. Abscess formation is a rare complication. Brucella endocarditis and neurobrucellosis cause most deaths.

Laboratory criteria

Presumptive diagnosis

- Rose Bengal test (RBT) for screening; positive tests to be confirmed by one of the tests mentioned below under *Confirmatory diagnosis* below;
- Standard agglutination test (SAT).

Confirmatory diagnosis

- Isolation of *Brucella* spp. from blood or other clinical specimen.
- A presumptive laboratory diagnosis based on detection of agglutinating antibodies (RBT, SAT) combined with detection of non-agglutinating antibodies

  through: – ELISA IgG test;
  – Coombs IgG.

  PCR and new rapid tests such as the lateral flow assay are yet to be accredited.

Case classification (humans)

- *Suspected:* a case that is compatible with the clinical description and is epidemiologically linked to suspected/ confirmed animal cases or contaminated animal products.
- *Probable:* a suspected case with presumptive laboratory diagnosis.
- *Confirmed:* a suspected or probable case with confirmatory laboratory diagnosis.

Surveillance

- *Rationale for surveillance:* surveillance is a key element for management of prevention and control programmes.
- *Recommended types of surveillance:* early case-based reporting by health care providers or laboratories to upper levels of the public health sector and to appropriate levels of the animal health sector; in endemic countries where investigation of all reported cases may not be feasible, a representative proportion of reported cases should be investigated routinely.

Recommended minimum data elements

Case-based data

- Case classification.
- Unique identifier, age, sex, geographical information and occupation.
- Aggregated data reporting
- Number of cases by case classification (probable/confirmed), age, sex, geographical area, occupation.

Recommended data analyses, presentation, reports

Graphs: number of probable/confirmed cases by month.
Tables: number of probable/confirmed cases by age, sex, month, and place.
Maps: number of probable/confirmed cases by place.

Performance indicators for surveillance

- Completeness and timeliness of reporting.
- Proportion of suspect, probable and confirmed cases.
- Number of investigations compared with number of cases.

Control activities

Case management

Doxycycline 100 mg twice a day for 45 days + streptomycin 1 g daily for 15 days. The main alternative therapy is doxycyclin 100 mg twice a day for 45 days + rifampicin 15mg/kg/day (600~900mg) for 45 days. Experience suggests that streptomycin may be substituted with gentamicin 5mg/kg/ daily for 7~10 days, but no study directly comparing the two regimes is currently available. Optimal treatment in pregnant women, neonates and children under 8 years has not yet been determined; for children there is experience with trimetoprim/sulfamethoxazole (co-trimoxazole) in combination with an aminoglycoside (streptomycin, gentamycin) or rifampicin.

Prevention

- Education to avoid consuming unpasteurized milk and milk derivatives.
- Barrier precautions for hunters and professionals at risk (butchers, farmers, slaughterers, veterinarians).
- Careful handling and disposal of afterbirths, especially in cases of abortion.
- Serological or other testing of animals; immunization of herds/flocks may be envisaged; elimination of infected herds/flocks.

Epidemics

Conditions under which epidemics may occur

Distribution of incriminated produce, usually raw milk or cheese from an infected herd/flock.

Management of epidemics

Identify common vehicle of infection; recall incriminated products, stop production and distribution unless pasteurization is introduced.

Drug-resistance monitoring

Not applicable.

Performance indicators for control activities

Number of new cases per
100 000 population over time.

Other aspects

Special considerations/other interventions. The most successful method for prevention and control of brucellosis in animals is vaccination. Control activities to be coordinated and shared between the public health and animal health sectors, who should ensure joint administrative arrangements to facilitate immediate cross-notification of cases, as well as coordination of joint investigations, control, and public health education programmes.

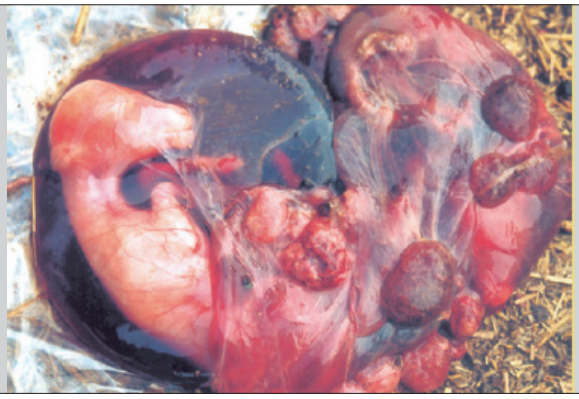
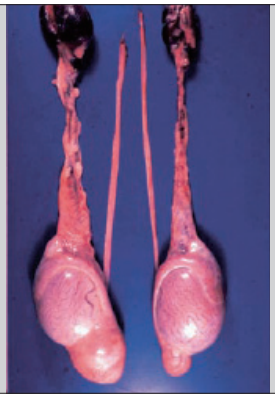


Figure 1
Brucellosis may produce abortion in goats or sheep at about the fourth month of pregnancy. *Brucella melitensis* is a major problem in many countries.

彩图 1
大约在怀孕第 4 个月，布鲁氏菌病可以导致绵羊或山羊的流产。在许多国家羊种布鲁氏菌是主要问题

Figure 2
Epididymitis (tail of epididymides) in a bull infected by *B. melitensis*.

彩图 2
公牛感染羊种布鲁氏菌的附睾炎（附睾的末端）



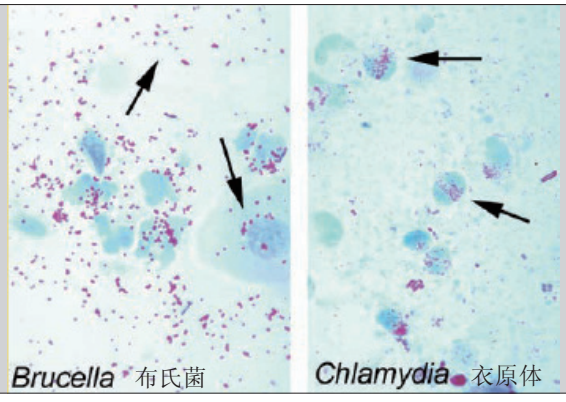


Figure 3
Stamp stain (modified Gimenez method) of vaginal swabs from aborted ewes. Note the differences between *Chlamydophila abortus* and *Brucella melitensis*.

彩图 3
流产母羊的阴道拭子印记染色（改良革兰染色方法）
注意流产衣原体与羊种布鲁氏菌的区别

Figure 4
Culturing: *Brucella* can be transmitted easily during laboratory work. Bacteriological analyses should always be performed under adequate protection in safety hoods.

彩图 4
培养：在实验室布鲁氏菌极易传播
应在安全防护服的充分保护下进行细菌学分析

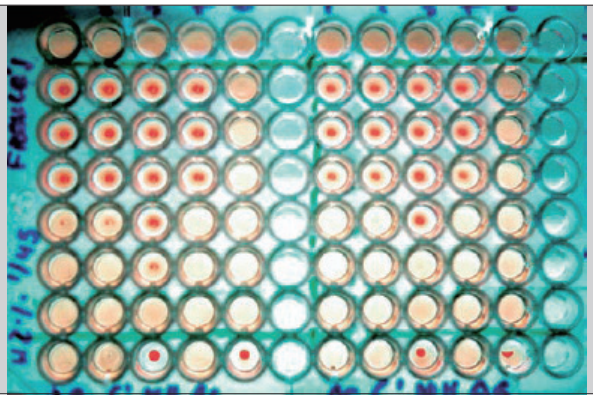
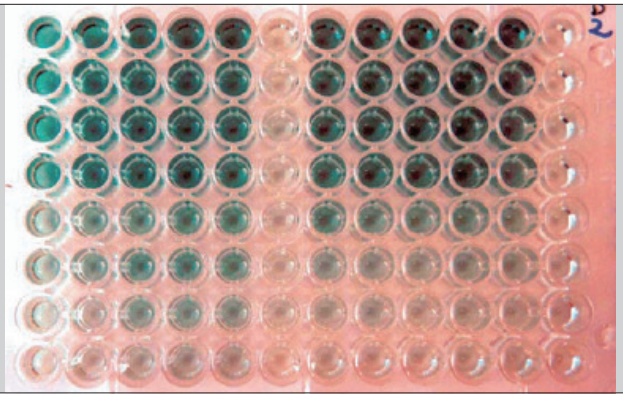


Figure 5
Complement fxation test is probably the most widely used serological test for the diagnosis of brucellosis in animals.

彩图 5
补体结合试验可能是诊断动物布鲁氏菌病应用最广泛的血清学试验

Figure 6
Indirect ELISA. Alternative screening (or confirmatory) test.

彩图 6
间接酶联免疫吸附试验
备选筛查（或确证）试验



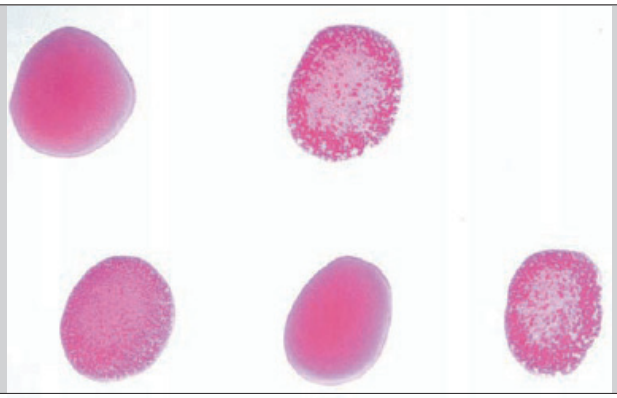


Figure 7
Rose Bengal plate test. The most widely used screening test.

彩图 7
虎红平板凝集试验
应用最广泛的筛查试验

Figure 8
A technician taking organs for bacteriological culture.

彩图 8
技术人员采集器官用于细菌学培养